

LOCAL AND REGIONAL ASPECTS OF HABITAT QUALITY JOINTLY
AFFECT THE BIODIVERSITY OF EPHEMERAL PONDS

by

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The biodiversity of local communities is likely affected by both local habitat quality and by the quality of the landscape surrounding the locality. In pond environments, habitat quality may be affected by the kind of leaf litter present because leaf species differ in the kind of habitat structure they provide and the rate at which they release nutrients into the water. Landscape quality could affect diversity as well; some landscapes provide a richer pool of potential colonists. In addition, the plant community surrounding ponds could alter the influence of habitat selection: animals may prefer to colonize ponds that have litter which matches that kind of litter typically produced by plants in the surrounding landscape (e.g. if organisms are adapted to the litter types in the habitats where they occur). We conducted a split-plot randomized block experiment to examine how both landscape and local scale properties, and their interaction, affect biodiversity within temporary pond communities in eastern NC. We manipulated both the kind of landscape in which artificial ponds were located (open-canopy grassland, pine forest, and hardwood forest) and the leaf species (sedge, pine, or maple) present in artificial ponds. Ponds were open to colonization by amphibians and aquatic insects during the summer of 2010. We surveyed organisms in the ponds on a monthly basis and did a complete census of each pond at summer's end.

The kind of plant community (landscape) had consistent, strong effects on biodiversity throughout the experiment: ponds in open-canopy landscapes supported more species and different kinds of species than ponds in forested systems. Litter type affected biodiversity in the monthly catch-and-release samples, with more species in sedge treatments than pine treatments. We also found evidence from the monthly samples to suggest biodiversity was enhanced to a greater extent in open canopy ponds when litter from open canopy environments was present but that biodiversity in closed canopy environments was affected less by the kind of litter present. Our results highlight the importance of the terrestrial matrix surrounding ponds on biodiversity within the ponds, and it could aid conservation efforts aimed at maintaining the unique biodiversity of temporary ponds.

LOCAL AND REGIONAL ASPECTS OF HABITAT QUALITY JOINTLY
AFFECT THE BIODIVERSITY OF EPHEMERAL PONDS

A Thesis

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CHAPTER 1: INTRODUCTION

One of the fundamental goals of ecology is to increase our understanding of what causes and maintains patterns of composition and abundance of species in natural communities.

Although a considerable amount of work has explored how habitat quality affects community composition (reviewed in Mortelliti, Amori, and Boitani, 2010), little work has explored the relative influence of different aspects of habitat quality that occur at different scales (Haynes et al., 2007). In addition, few studies explore whether habitat quality is assessed similarly in different contexts (e.g. with different sets of species). It is essential to determine how local and regional aspects of habitat quality jointly affect community dynamics if we are to advance predictive theory and develop better strategies for conservation of biodiversity (Frey et al., 2011; Schooly and Branch, 2011; Mortelliti, Amori, and Boitani, 2010).

The metacommunity concept provides a unified framework for describing community dynamics by including processes that operate at both local and regional scales (Wilson, 1992; Leibold et al., 2004). In this framework, multiple local communities are linked regionally via dispersal between patches. Community structure within patches arises due to dispersal and colonization dynamics at the regional scale and from local biotic and abiotic interactions that regulate the persistence of species in the community. However, it remains unclear whether habitat quality is important in metacommunity dynamics relative to patch size and isolation (Moilanen and Hanski, 1998; Mortelliti, Amori, and Boitani, 2010). In addition, more empirical work is needed that shows the relative influence of local and regional factors and how they interact to determine community structure (Werner et al., 2009).

Lentic freshwater communities such as ponds are often viewed in a metacommunity context: individual ponds are patches of aquatic habitat that are isolated from each other by a

terrestrial matrix, but linked to each other by dispersal of species with both aquatic and terrestrial life stages (Wilbur, 1997; Binckley and Resetarits, 2007; Werner et al., 2009). Many local factors at the scale of an individual pond affect community structure, such as species interactions, resource levels, and hydroperiod (Shurin, 2001; Kneitel and Miller, 2003; Van Buskirk, 2005). Colonization dynamics at the regional scale can also have major effects on pond communities. For example, the order in which species colonize a pond from the region can have important effects on relative rates of resource acquisition between species; these “priority effects” can alter the outcome of competitive interactions and population persistence (Alford and Wilbur, 1985). Many studies of the factors regulating community structure in ponds include multiple processes that operate at both local and regional scales. For example, many habitat selection studies have shown that colonization rates in ponds are often mediated by a selection process in which species prefer ponds that have local characteristics that maximize their fitness. For example, aquatic insects and ovipositing amphibians have been shown to preferentially colonize ponds that lack fish predators, ponds that lack conspecific competitors, and ponds imbedded in more open-canopy terrestrial plant communities, where productivity is higher (Wellborn et al., 1996; Resetarits, 2001; 2005; Binckley and Resetarits, 2007). Ponds provide an ideal model system to address the relative and interactive importance of factors at different scales during community assembly, however, the role of local habitat quality and landscape context on the dynamics of this system is seldom explored simultaneously.

One local factor that may affect community structure in ponds is the type of leaf litter present in the pond. Leaf litter is a common form of detritus in many ecosystems, and it is a major source of habitat, energy, and nutrients (Moore et al., 2004). Leaf litter releases nutrients as it decomposes, and is expected to be important for the productivity of the basal trophic levels

in aquatic systems: bacteria, fungi, and algae (Tuchman et al., 2002; Gessner et al., 2007; Stoler and Relyea, 2011). Leaf litter may have effects on community structure at higher trophic levels by altering population sizes and food web dynamics in these systems (Rubbo and Kiesecker, 2004; Williams et al., 2008). Leaf litter can also be an important energy source in aquatic systems where primary productivity is low (Polis et al., 1997; Wallace et al., 1997; Rubbo et al., 2006; Batzer and Palik, 2007; Stoler and Relyea, 2011). Leaf litter from different plant species can vary in quality or accessibility to detritivores, depending on (1) the amount and proportion of essential nutrients, such as nitrogen and phosphorus, (2) the amount of fiber or lignin, and (3) the amount of chemical defenses in the leaves (Webster and Benfield, 1986; Ostrofsky, 1997; Swan and Palmer, 2006). The quality of leaf litter can affect the consumption rates and growth of herbivores and detritivores (Herbst, 1982; Going and Dudley, 2008; Stoler and Relyea, 2011). The diversity and abundance of invertebrates colonizing stream microhabitats and tree holes also seems to increase with increasing litter quality as well (Egglishaw, 1964; Short et al., 1980; Yanoviak, 1999; Richardson et al., 2004; Palik et al., 2006). However, some studies show no relationship between leaf litter quality and the diversity, abundance, or growth of invertebrate colonizers (Imbert and Pozo, 1989; Batzer and Wissinger, 1996; Leroy and Marks, 2006; Batzer and Palik, 2007; Reiskind et al., 2009). This inconsistency suggests that the influence of detritus quality on community structure is complex and not fully understood. In addition, leaf litter effects on community structure have been relatively well-studied within lotic systems, especially headwater streams, but the importance of litter in ponds is seldom investigated (Williams et al., 2008; Stoler and Relyea, 2011).

The type of leaf litter in a pond depends on the structure of the surrounding plant community. For example, some aquatic systems receive allochthonous leaf litter inputs from

terrestrial-based plants while other systems rely on autochthonous input of litter from within the aquatic environment (Rounick et al., 1982; Cummins et al., 1989; Richardson et al., 2004; Palik et al., 2006). In addition, the abundance of different types of leaf litter in ponds will depend on the relative abundance of taxa in the surrounding plant community (Rubbo and Kiesecker, 2004). However, the plant community surrounding a pond is also correlated with many other biotic and abiotic factors that could also affect community structure within ponds. Determining the relative importance and interactions of the surrounding plant community and local leaf litter types requires the simultaneous experimental manipulation of both factors, but these manipulations are seldom performed (McCauley, 2007; Williams et al., 2008).

Systems of ponds are imbedded in a terrestrial matrix that could affect both local dynamics within ponds and regional processes such as colonization and habitat selection. The specific type of plant communities surrounding ponds may have several major effects on aquatic community dynamics. In addition to differences in leaf litter, different plant communities show different levels of canopy cover. This gradient of canopy cover is associated with variation in other abiotic and biotic factors important to aquatic systems including solar input, dissolved oxygen, water temperature, primary production, and predator types (Werner and Glennemeier, 1999). Field surveys typically find lower abundance and diversity of aquatic taxa in more closed-canopy areas (Skelly et al., 1999; Batzer et al., 2000, Halverson et al., 2003, Werner et al., 2007). Experiments by Binckley and Resetarits (2007; 2009) suggest that these observed differences in community structure across the gradient of canopy cover are explainable mostly by habitat selection, although post-colonization processes (such as reduced survivorship in closed-canopy areas) likely drive the pattern as well (Werner and Glennemeier, 1999; Rubbo and Kiesecker, 2004; Schiesari, 2006; Williams et al. 2008).

CHAPTER 2: THE EXPERIMENT

Using aboveground mesocosms (wading pools) as artificial ponds, we altered the types of leaf litter within the mesocosms and the type of dominant plant community surrounding mesocosms to determine the combined influence of these two factors on community assembly. While the two factors are related, they operate naturally at different scales (within ponds versus across multiple ponds) and may have interactions that are difficult to predict without their independent manipulation. For example, if the distribution of aquatic organisms varies with different litter types, any differences in aquatic community structure due to the surrounding plant community may be further exaggerated when the leaf litter in the pond matches the litter type in the surrounding plant community. We predict that, due to increased productivity, mesocosms within more open-canopy plant communities will support a greater abundance and diversity of colonizers. In addition, we predict that mesocosms with higher-quality litter will support more abundance and diversity of colonists, independent of the surrounding plant community.

Methods

Study System

This study will test the effects of leaf litter type and plant community on the diversity of temporary pond communities. Temporary ponds have a cyclic hydroperiod with wet and dry stages, usually controlled by seasonal rainfall and evapo-transpiration (Kirkman et al., 1999). The hydroperiod of temporary ponds can vary; some ponds only fill for a few months of the year, while others can hold water for most of the year (Batzer and Wissinger, 1996). These ponds are ubiquitous in the coastal plain of the southeastern United States and can be important components of regional biodiversity (Kirkman et al., 1999). Temporary ponds often support a unique community of plants and animals that have adaptations to seasonal drying, such as

drought-resistant stages and rapid development (Batzer and Wissinger, 1996; Kirkman et al., 1999).

Temporary ponds usually lack fish, and may therefore contain a higher diversity of invertebrates and larval amphibians that are released from predation (Kirkman et al., 1999). Many amphibians are temporary pond specialists that will not breed in ponds in which fish are present, and therefore, the amphibian assemblage in temporary ponds may contain species that are not found in more permanent bodies of water (Snodgrass et al., 2000).

Temporary pond systems are especially tractable for use in population and community ecology experiments because they have discrete boundaries and are relatively simple systems (De Meester et al., 2005). In addition, mesocosms (such as above-ground plastic wading pools) can be used to recreate temporary pond communities that approach the complexity of natural systems, and because temporary ponds usually dry in a yearly cycle, experiments can be run realistically in one season (Wilbur, 1997). Mesocosms have been used extensively to study species interactions, colonization, and habitat selection in temporary pond taxa (Wilbur, 1997; Binckley and Resetarits, 2005; Resetarits, 2005). Finally, mesocosms are ideal for experimental studies; they allow for increased replication and treatment combinations relative to field studies (Williams, 2008), without the problem of confounding variables like differences in hydrology and other sources of variability in natural systems (Chalcraft et al., 2005).

We focused on a subset of temporary pond taxa: macroinvertebrates (e.g. insects) and amphibians. Temporary ponds can support large numbers and a high diversity of insects and amphibians (Kirkman et al., 1999), and these organisms can often disperse and colonize new areas very efficiently (Batzer and Wissinger, 1996; Resetarits, 2005). Most pond insects will readily colonize man-made water sources, and have the ability to colonize above-ground

mesocosms aerially. Some pond amphibians, such as Hylid frogs, will readily climb into and oviposit in above-ground mesocosms as well (Binckley and Resetarits, 2005; Resetarits, 2005). Insect and amphibian communities can develop rapidly, and therefore provide a good opportunity to explore the development and structure of communities in a short-term study (Wilbur, 1997; Binckley and Resetarits, 2005; Resetarits, 2005).

Experimental Design

We conducted a split-plot randomized block experiment to examine how both landscape and local scale properties affect biodiversity within temporary pond communities in eastern NC. We manipulated both the kind of plant community in which mesocosms were located and the leaf species present in mesocosms. At the landscape scale, we focused on three different treatments for the plant community manipulations: open-canopy grassland, pine-dominated forest, and hardwood-dominated forest. These three types of plant communities are very common and represent a typical secondary succession and canopy-cover gradient in NC (Bormann, 1953). Open-canopy communities exist in areas of major disturbance, such as mowed fields, and are usually dominated by herbaceous vegetation such as graminoids and forbs. Old fields are typically invaded by pine trees within 5 years, and canopy cover increases dramatically within 10 years when the system becomes dominated by pine trees. Eventually, hardwoods will invade and replace pines as the dominant tree species in mature forests (Bormann, 1953).

For local litter type manipulations, we focused on three litter species as our treatments: beaksedge (*Rhynchospora inundata*), red maple (*Acer rubrum*), and loblolly pine (*Pinus taeda*). These litter types represent a wide gradient of litter quality (based on their breakdown rates, see Webster and Benfield, 1986) from low (pine) to high quality (sedge). They also represent

differences in the kinds of litter found in natural ponds along a successional gradient. For example, aquatic macrophyte litter, such as *Rhynchospora*, is characteristic of ponds in open-canopy, early-successional plant communities (Williams, 2008). Loblolly pine is very prevalent in mid-successional forests in Eastern NC, and it is commonly used in the commercial timber industry (Bormann, 1953; Goodman et al., 2006). Red maple is a common deciduous species in mid- to late-successional forests in Eastern NC (Peroni, 1994), and it is a facultative wetland species that is prevalent around many freshwater aquatic ecosystems (Rheinhardt et al., 1998). There is some evidence that mature hardwood forests in the eastern U.S. are shifting from oak-dominated forest to red maple-dominated, which may affect the structure of temporary pond communities within those forests (Rubbo and Kiesecker, 2004). All three leaf litter types were collected in March. The litter was air-dried indoors and weighed before it was added to the mesocosms.

Field mesocosms were constructed from wading pools 1.5 meters in diameter and 0.29 meters tall (volume approximately 400 L). We employed a split-plot design, with multiple leaf litter (subplot) treatments nested within each replicate of the plant community (whole plot) treatment. A total of 12 field sites were used: 4 independent sites for each of the three plant community treatments. These sites included locations in Pitt County, Craven County, and Beaufort County, NC. Three mesocosms, one for each leaf litter treatment, were established at each site. The mesocosms were arranged approximately 1 meter apart from each other in a triangular formation. Sites were chosen so that blocks could be formed, with representatives of each plant community treatment in close proximity (Figure 1). All setup and sampling procedures were performed on a block-by-block basis in order to account for the possible effects of temporal variability in methods.

Mesocosms were established in May. We filled each mesocosm with well water, and a random number generator was used to assign litter treatments to each mesocosm. We then added 300 grams of the assigned leaf litter type to each mesocosm. Each mesocosm also received a mesh bag containing a small amount of litter of known weight (matching the litter assigned to that mesocosm) in order to measure the amount of mass loss as a result of decomposition. Finally, we added a strip of flagging tape to each wading pool: we measured periphyton growth at the end of the experiment by scraping the algae off of a 10 centimeter portion of each strip of flagging tape. Mesocosms were left open to colonization by insects and amphibians for the duration of the experiment.

Sampling Methods

Macroinvertebrates and amphibians in each mesocosm were sampled once in June and again in July using a non-destructive, catch-and-release technique: organisms were sampled with two sweeps of a 20 by 30 centimeter fish net (1 mm mesh size) and captured individuals were counted and identified to the lowest taxonomic resolution possible in the field, then returned to the pool. This procedure allowed us to sample individuals through time without altering community assembly. Nets were deep enough that the full range of depth in the mesocosm was captured in each sweep. These catch-and-release samples consisted of two sweeps from each mesocosm: one across the middle of the diameter, and one along a random 1/3 of the circumference. This sweeping procedure captured individuals at all depths, at the sides, and in the middle of the mesocosms.

The experiment ended in August. The mesocosms were destructively sampled using a much more intensive sweeping procedure: 20 by 30 centimeter fish nets were used to sweep from the pool until no insects or amphibians were found in ten consecutive sweeps. The sweep

contents were placed into a water-filled Rubbermaid container. Leaf litter was checked for organisms and then discarded. Amphibians were identified in the field and released to natural water sources. The remaining macroinvertebrates and small pieces of leaf litter were collected in a sieve and preserved in 70% ethanol. We picked through these samples under a dissection microscope while counting and identifying all macroinvertebrates.

From our measurements of community composition in the catch-and-release samples and destructive samples, we calculated species richness (number of unique species) and total community abundance (total number of individuals of all species) in each sample. We measured leaf decomposition, phytoplankton abundance, and periphyton abundance at the end of the experiment. Leaf decomposition was measured by air-drying the leaves in the leaf packs, determining their final mass, and subtracting from their initial mass in order to calculate the proportion of mass that was lost over the course of the entire experiment. Phytoplankton was obtained by taking a 550 milliliter water sample from each mesocosm, while periphyton was obtained by scraping algae off of a 10 centimeter strip of the flagging tape from the mesocosm and rinsing the algae with water into a 100 milliliter sample cup. Both types of algal samples were processed the same way to determine chlorophyll abundance. Each sample was filtered through a glass fiber filter with a pore size of 1.2 micrometers. The filters were frozen before being processed, and during processing, kept in dark, cool conditions as much as possible to prevent chlorophyll degradation. Each filter was homogenized in a tissue grinder, and then chlorophyll was extracted using 90% acetone. Chlorophyll *a* abundance was determined using a phaeophytin-corrected spectrophotometric analysis (APHA, 1995).

We became interested in light availability at our field sites after gathering preliminary results from the experiment. We went back to the 12 sites on a sunny day in June 2011 and

measured available light in order to determine the effect of the plant community treatment on light levels. We took three measurements at each site, and used the average from each site in the analysis.

One mesocosm was punctured and lost nearly all of its water before the August sampling, and was therefore excluded from all August analyses. In addition, several of our leaf packs and periphyton flagging strips were found damaged and outside the water (most likely disturbed by raccoons), and were therefore excluded from the analyses.

Analyses

We analyzed our data using both univariate and multivariate analyses. Univariate analyses were performed in SAS for Windows version 9. We used a two-way ANOVA to determine the effects of plant community and block on light levels. Pairwise comparisons were achieved using a post-hoc REGW test. For the catch-and-release samples, we used a repeated measures split-plot ANOVA (SAS Proc Mixed) to detect differences in species richness and total community abundance due to litter type, plant community, time (month), litter type \times plant community interaction, month \times litter type interaction, month \times plant community interaction, and the three-way month \times litter type \times plant community interaction. Additional split-plot ANOVAs were used to detect treatment differences in August species richness, August total community abundance, leaf decomposition, periphyton abundance, and phytoplankton abundance due to litter type, plant community, and the litter type \times plant community interaction. The Satterthwaite procedure was used to calculate degrees of freedom in all split-plot ANOVAs. When the overall ANOVA indicated a significant effect, we used the Tukey's HSD post-hoc test to determine pairwise differences between treatments.

Multivariate analyses, which we used to compare community structure among our treatments, were performed in Primer version 6. Primer emphasizes differences in relative abundance distributions between communities by creating a Bray-Curtis Dissimilarity matrix in which the abundance distribution in each sample is compared to all of the other samples. Each comparison is assigned a dissimilarity score based on how different the two communities are. This matrix of dissimilarity scores can be used to create ordination plots using non-metric multidimensional scaling (NMDS). We created NMDS plots for each sampling date (June, July, and August). In addition, using permutation methods, Primer allows groups of communities to be compared statistically. We used two such tests: PERMANOVA (which like a regular ANOVA incorporates differences within and among groups in dissimilarity values), and PERMDISP (which compares the extent of variability within treatments). PERMANOVA allows you to statistically test differences you can see in the NMDS plots based on the location of group centroids and the amount of variation around those centroids. PERMDISP allows you to detect differences in variability around the centroids only.

We used a split-plot PERMANOVA and PERMDISP to provide a robust comparison of community structure among our treatments for both the catch-and-release and the destructive samples. We explored the effects of litter type, plant community, and the litter type \times plant community interaction. When the analysis indicated an overall treatment effect, we performed pairwise comparisons to determine which treatment groups were different. Primer uses uncorrected t-tests in its pairwise comparisons, so we adjusted the resulting p-values by controlling for the False Discovery Rate. Once we knew what treatments differed, we employed another analysis, Simper, which determines the importance of each taxon in its contribution to similarities within treatments and differences among treatments.

Results

Overall, more than 68,000 individuals from over 70 species were counted and identified, including the catch-and-release samples during the course of the experiment.

Species Richness

Species richness in the catch-and-release samples (Figure 2) was not affected by month ($F_{1,27}=1.43$, $p=0.2417$), month \times plant community ($F_{2,27}=0.02$, $p=0.9811$), month \times litter type ($F_{2,27}=0.75$, $p=0.4841$), or month \times plant community \times litter type ($F_{4,27}=0.59$, $p=0.6711$). There was a significant effect of litter type on species richness ($F_{2,18}=4.13$, $p=0.0333$). Pairwise comparisons revealed that mesocosms with sedge litter supported significantly more species than mesocosms containing pine litter (Tukey's HSD: $p=0.0291$). The plant community treatment also significantly affected species richness in the catch-and-release samples ($F_{2,9}=8.42$, $p=0.0087$); mesocosms in open-canopy areas supported more species than mesocosms in both pine forests (Tukey's HSD: $p=0.0348$) and hardwood forests (Tukey's HSD: $p=0.0089$). Species richness was also affected by the leaf litter \times plant community interaction ($F_{4,18}=3.02$, $p=0.0453$). This interaction seems to be driven by stronger litter effects in open-canopy plant communities (Figure 2b).

In the August census samples, species richness (Figure 3) was not affected by litter type ($F_{2,17.2}=1.70$, $p=0.2116$). The surrounding plant community had significant effects ($F_{2,9.09}=11.51$, $p=0.0032$): open-canopy plant communities had significantly higher species richness than both pine and hardwood-dominated forests (Tukey's HSD: $p<0.008$). The plant community \times litter type interaction, however, did not significantly affect species richness in August ($F_{4,17}=1.15$, $p=0.3673$).

Total Community Abundance

Total community abundance in the catch and release samples (Figure 4) was not affected by month ($F_{1,27}=2.07$, $p=0.1615$), month \times litter type ($F_{2,27}=1.12$, $p=0.3422$), litter type \times plant community ($F_{4,18}=0.62$, $p=0.6547$), or month \times plant community \times litter type ($F_{4,27}=0.33$, $p=0.8531$). There was a trend for an effect of litter type ($F_{2,18}=3.21$, $p=0.0643$) that seems to be driven by higher total community abundance in mesocosms with sedge litter compared to mesocosms with pine or maple litter (Figure 4a). There was also a trend for an effect of plant community on total community abundance ($F_{2,6}=4.31$, $p=0.0690$), with lower abundance on average in mesocosms within open-canopy plant communities versus those in pine-dominated and hardwood-dominated forests. However, this trend is driven primarily by the strong effect of plant community on total community abundance in June (Figure 4b), as evidenced by the significant month \times plant community interaction ($F_{2,27}=6.97$, $p=0.0036$). Pairwise comparisons indicate that mesocosms in open-canopy plant communities had significantly lower abundance relative to mesocosms in pine- and hardwood-dominated forests, but only in June (Tukey's HSD: $p<0.007$).

In the August census samples, total community abundance (Figure 5) was not affected by litter type ($F_{2,17.6}=1.10$, $p=0.3535$), plant community ($F_{2,9.25}=0.89$, $p=0.4413$), or the plant community \times litter type interaction ($F_{4,17.6}=1.34$, $p=0.2936$).

Multivariate Community Composition

Non-metric multidimensional scaling plots are shown in Figures 6 and 7. PERMANOVA revealed that litter type did not affect multivariate community structure at any point during the experiment (Figure 7, panels b, d, and f), although there was a trend for a litter effect in June (June: Pseudo- $F_{2,18}=1.74$, $p=0.0737$; July: Pseudo- $F_{2,18}=1.27$, $p=0.2413$; August:

Pseudo- $F_{2,17}=1.06$, p=0.3764). The trending litter effect in June seems to be driven by different community structure in mesocosms with maple litter compared to pine or sedge litter (Figure 7b). Variability in community structure, as compared with PERMDISP, was also not significantly affected by litter treatments at any point during the experiment (June: Pseudo- $F_{2,33}=0.07$, p=0.9562; July: Pseudo- $F_{2,33}=0.16$, p=0.8907; August: Pseudo- $F_{2,32}=0.21$, p=0.8439).

In contrast, plant community treatments affected multivariate community structure throughout the experiment. PERMANOVA revealed significant overall effects of plant community at each sampling point (June: Pseudo- $F_{2,6}=5.82$, p=0.0005; July: Pseudo- $F_{2,6}=4.53$, p=0.0016; August: Pseudo- $F_{2,6}=4.09$, p=0.0044). Pair-wise differences indicate that mesocosms in open-canopy plant communities had significantly different community structure relative to the aquatic communities in pine- and hardwood-dominated forests after controlling for the False Discovery Rate (p<0.05 in all cases; Figure 7, panels a, c, and e). In addition, there was a weak trend for different community structure in hardwood vs. pine sites in June (FDR-adjusted p=0.0942; Figure 7a). This trend seems to be driven by higher proportions of mosquito larvae (Culicidae) and *Hyla* tadpoles and lower proportions of midge larvae (Chironomidae) in hardwood sites relative to pine sites (see Simper results, Figure 9c).

PERMDISP, like PERMANOVA, also revealed significant plant community effects throughout the experiment (June: Pseudo- $F_{2,33}=29.50$, p=0.0001; July: Pseudo- $F_{2,33}=12.16$, p=0.0005; August: Pseudo- $F_{2,32}=4.71$, p=0.0186). In June (Figure 7a), pairwise differences show significantly different amounts of variability among all three plant communities (FDR adjusted p<0.044 in all cases): aquatic communities in hardwood forests showed the least amount of variability, communities in pine forests showed intermediate variability, and communities in open-canopy plant communities showed the highest variability. In July (Figure 7c), aquatic

community structure in hardwood forests was less variable than communities in pine forests and open-canopy plant communities (FDR-adjusted $p=0.0005$ in both comparisons). In August (Figure 7e), aquatic community structure was less variable in hardwood forest sites than in pine forest sites (FDR-adjusted $p=0.0300$).

The plant community \times litter type interaction (Figure 6) produced no detectable difference in multivariate community structure using PERMANOVA (June: Pseudo- $F_{4,18}=1.22$, $p=0.2387$; July: Pseudo- $F_{4,18}=0.97$, $p=0.4892$; August: Pseudo- $F_{4,17}=0.96$, $p=0.5018$). Significant responses to the plant community \times litter type interaction were detected using PERMDISP in June and July (June: Pseudo- $F_{8,27}=8.29$, $p=0.0006$; July: Pseudo- $F_{8,27}=4.44$, $p=0.0298$; August: Pseudo- $F_{8,26}=1.86$, $p=0.2211$). No significant differences were detected in pairwise comparisons after controlling for the False Discovery Rate ($p>0.08$ in all cases), but several trends (FDR adjusted $p<0.09$) indicate that the significant overall plant community \times litter type interaction in June and July is driven by higher variability among litter treatments in the open-canopy plant community treatments relative to forested treatments (Figure 6, panels a and b).

The Simper procedure was used to determine the taxa responsible for similarities within and differences among plant communities at each sampling date. The taxa that were responsible for driving similarities within each plant community are shown in Figure 8. Mesocosms in open-canopy plant communities (Figure 8a) tended to be characterized by high abundances of Chironomid larvae, *Laccophilus* beetles, Hydrophilid beetles (*Tropisternus* and *Berosus*), and in later months, Libellulid naiads. Mesocosms in pine- and hardwood-dominated forests (Figure 8b and 8c) were characterized by high abundances of Chironomid larvae, Culicid larvae, and *Hyla* tadpoles.

The taxa that were responsible for driving differences among plant communities are shown in Figure 9. Differences between community composition in open-canopy areas versus pine- and hardwood-dominated forests (Figures 9a and 9b), which were significantly different based on PERMANOVA, were primarily driven by Chironomids, Culicids, and *Hyla* tadpoles (more abundant in forested areas), and *Berosus* beetles (more abundant in open-canopy areas). There was a trend for a difference in community structure between pine-dominated and hardwood-dominated sites (Figure 9c) in June. Simper results suggest that this difference is driven by Chironomids (which were more abundant in pine-dominated forests), Culicids, and *Hyla* tadpoles (which were both more abundant in hardwood-dominated forests).

Leaf Decomposition, Light, and Algae Abundance

Decomposition (Figure 10) was affected by both litter type and plant community, but not by their interaction (Litter: $F_{2,15.6}=17.73$, $p<0.0001$; Plant community: $F_{2,10.3}=4.85$, $p=0.0327$; Plant community \times Litter type: $F_{4,15.3}=1.93$, $p=0.1570$). Tukey's HSD revealed that pine litter decomposed significantly less than maple or sedge litter ($p=0.0009$ and $p<0.0001$ respectively; Figure 10a). In addition, pairwise differences indicate that there are strong trends for decomposition to be slower in open-canopy plant communities relative to pine- and hardwood-dominated forests ($p=0.0529$ and $p=0.0602$ respectively; Figure 10b), which drive the significant overall plant community effect on leaf decomposition.

Light levels (Figure 11) differed significantly among our plant community treatments ($F_{2,6}=49.58$, $p=0.0002$). REGW groupings indicate that open-canopy sites had significantly higher light levels than pine-dominated and hardwood-dominated sites. However, light levels were not affected by block ($F_{3,6}=1.48$, $p=0.3130$).

Algae standing stocks (Figure 12) were not affected by litter type (Phytoplankton: $F_{2,17,1}=2.27$, $p=0.1331$; Periphyton: $F_{2,22,1}=0.80$, $p=0.4623$), nor the plant community \times litter type interaction (Phytoplankton: $F_{4,17,1}=1.42$, $p=0.2693$; Periphyton: $F_{4,22,1}=1.74$, $p=0.1760$; Figure 12a and 12b). However, phytoplankton and periphyton abundances were significantly affected by plant community (Phytoplankton: $F_{2,5,77}=5.56$, $p=0.0451$; Periphyton: $F_{2,22,1}=25.37$, $p<0.0001$). Tukey's HSD showed that mesocosms in hardwood-dominated forests had significantly less periphyton abundance than mesocosms in open-canopy and pine-dominated plant communities (Figures 12c and 12d; $p<0.0001$ in both comparisons). Phytoplankton abundance was lower in hardwood forests relative to open-canopy plant communities (Tukey's $p=0.0474$).

Discussion

Plant Community

The plant community surrounding ponds had very strong effects on colonizing amphibians and macroinvertebrates, independent of leaf litter effects. The most substantial differences in species richness and abundance were shown between open-canopy communities and forested communities, with few differences in the aquatic communities detected between pine-dominated and hardwood-dominated forests. Total community abundance (number of individuals of all species) was affected by plant community with higher abundance in pine and hardwood sites relative to open-canopy sites, but only in June. This abundance effect may be driven by differences in the relative proportion of prey and predator species, as evidenced by the Simper results from the June sampling point. In June, most forested areas had very few predator species present; those mesocosms were characterized by large numbers of Chironomid and Culicid midge larvae, as well as Hylid tadpoles. Open-canopy areas, in contrast, already had

many predaceous water bugs, diving beetles, and dragonfly larvae. This difference in composition could drive the difference in total community abundance that was detected in June, and explain why there was no effect of plant community for the rest of the experiment, once predaceous taxa colonized the mesocosms in forested areas.

Species richness was much higher overall in the ponds embedded in open-canopy plant communities, a result which mirrors the associations found in surveys of natural ponds (Skelly et al., 1999; Batzer et al., 2000, Halverson et al., 2003, Werner et al., 2007). The cause of the relationship between species richness and canopy-cover can either be driven by differential colonization, or by differences in post-colonization extinction rates. Manipulative, short-term studies show that the relationship between canopy cover and species richness can be primarily driven by colonization behavior in both aquatic insects and amphibians (Binckley and Resetarits 2007; 2009). Canopy cover has also been shown to affect the performance of amphibians, often with reduced survivorship in closed-canopy areas due to lower algal resources and lower dissolved oxygen in those areas (Werner and Glennemeier, 1999; Skelly et al., 2002; Halverson et al., 2003); however, the effect of canopy cover on macroinvertebrate performance is still unclear (Batzer et al., 2004). The pattern of increasing species richness over time in our experiment, coupled with the strong effect of canopy cover even at the earliest sampling date, suggests that preferential colonization of open-canopy ponds is the more likely cause of our observed pattern in species richness.

Binckley and Resetarits (2007) argue that behavioral responses to canopy cover are so strong that many aquatic taxa may simply avoid closed-canopy ponds altogether. Of the 74 species present in our August samples, 19 species (25.7%) were found only in forested communities, 29 species (39.2%) were found only in open-canopy communities, and 26 species

(35.1%) were found in both forested and open-canopy plant communities. There was also no consistent preference for open sites in the species that occurred in both open- and closed-canopy ponds. Our results suggest that the communities in closed-canopy and open-canopy ponds are comprised of relatively distinct sets of species. Therefore, diversity differences along the gradient of canopy cover could arise in large part simply because the assemblage of potential colonists is more diverse in open-canopy plant communities relative to the potential colonists in forested areas. Hence, as postulated by Binckley and Resetarits (2007; 2009), closed-canopy ponds may be avoided by many species because they are not considered habitat at all, rather than being perceived as low-quality habitat that would eventually be suitable for colonization once higher-quality ponds were saturated with individuals (as predicted by the Ideal Free Distribution, *sensu* Fretwell and Lucas, 1986).

The reason remains unclear regarding why open-canopy areas have a more diverse assemblage of animal colonists. The main hypothesis that has been suggested for the association between species richness and canopy cover is a diversity-productivity argument: in low productivity aquatic systems, the increase in productivity as canopy cover decreases is expected to cause an increase in biodiversity (Abrams, 1995; Yanoviak, 1999; Skelly, 1999). Increased productivity in open-canopy environments is caused by more light availability, higher temperatures, and higher dissolved oxygen (Werner and Glennmeier, 1999). We showed that, as expected, light levels were significantly higher in our open-canopy sites relative to pine- and hardwood-dominated forests. Our measures of algae standing stocks (Figure 9) show high abundance of algae in both open-canopy communities and pine-dominated forests; however, standing stocks may not be a good measure of algal productivity (i.e. growth rate) if there is temporal variation in algal growth or if the increasing algal standing stocks results in more

herbivory. Also, algae is not the only basal resource in ponds; many macroinvertebrates may be just as dependent on fungal and bacterial production relative to algal production (Tuchman et al., 2002; Gessner et al., 2007; Stoler and Relyea, 2011). Still, productivity of fungi and bacteria are likely increased in open-canopy areas, with higher temperatures and dissolved oxygen, as well (Tuchman et al., 2002; Gessner et al., 2007). Therefore, the relationship between productivity and diversity may drive the pattern of increasing species richness in open-canopy communities.

Surrounding plant community also had effects on leaf decomposition, with trends for less decomposition in open-canopy plant communities relative to forested communities. This result may seem counter-intuitive, because decomposition has been shown to increase with temperature and with biodiversity of macroinvertebrates, two factors that were higher in open-canopy plant communities in our experiment (Ferreira and Chauvet, 2011, Hobbie, 1996; Jonsson and Malmqvist, 2000). In addition, litter decomposition in streams has been shown to be slower in experimentally shaded sites versus unaltered sites (Lagrule et al., 2011).

One hypothesis for our result is that our leaf decomposition data is affected by differences in periphyton growth on the leaves—open-canopy areas could have more algae that contribute to the mass of the leaves, resulting in less perceived decomposition. However, this hypothesis can be argued against using two lines of evidence. First, our periphyton data show similar standing stocks in both open-canopy sites and pine forest sites, but only open-canopy areas seem to have less decomposition. Second, if an algal growth is contributing to the weight of the leaves, you might expect a significant litter type \times plant community interaction effect on decomposition, for our litter types differ greatly in surface area. This difference in surface area should affect how strongly decomposition depends on the surrounding plant community if

differences in algal growth on the litter are causing the plant community effect on decomposition (we detected no such interaction).

Another explanation for faster litter decomposition in closed-canopy aquatic systems relative to open-canopy systems is a shift from a detritus-based food web in closed-canopy areas to an algae-based food web as canopy cover decreases (Vannote et al. 1980, Earl et al., 2011). Organisms may be more dependent on leaf litter as an energy resource in closed-canopy areas where primary productivity is low. This shift in food web resources may lead to the prediction of context-specific leaf litter effects. For example, if species in closed-canopy areas are more reliant on the leaf material as an energy source, one might predict organisms in those areas to be more selective, resulting in a stronger response to litter quality in forested areas. Alternatively, if the effects of litter decomposition on algal growth are more important, you may expect stronger litter responses to litter quality in open-canopy areas that are more reliant on primary production. We found a significant interaction between litter type and plant community in the catch-and-release samples showing stronger litter effects in open-canopy communities relative to closed-canopy communities. Therefore, because litter types seem to affect community structure more strongly in open-canopy areas with algae-based food webs, the importance of litter in pond animal communities may be driven primarily by its effect on algal growth.

The strong effect of surrounding plant community on community structure found in our study may be altered by other factors in natural ponds. First, the effects of plant community were found to be driven by canopy cover, which in our study was either completely open or completely closed. While pond surveys have demonstrated direct, positive associations between species richness and decreasing canopy cover (Skelly et al., 1999; Batzer et al., 2000, Halverson et al., 2003, Werner et al., 2007), more experimental work is needed to determine how pond

communities are affected across the entire gradient of canopy cover (Binckley and Resetarits, 2009). Seasonality is another factor that potentially affects the relationship between colonization behavior and canopy cover. This experiment was conducted after leaf emergence during the summer, when canopy cover is at its maximum. Binkley and Resetarits (2009) suggest that ponds in forested areas may become less appealing and more difficult to locate as leaves emerge and less light reaches the surface of ponds. Additional work is needed to determine whether the species diversity of ponds in forested areas continues to be lower once the leaves of deciduous trees have senesced.

Leaf Litter

Local leaf litter types also had significant effects on community composition. However, the litter effects tended to be weaker and more variable than the plant community effects. Total community abundance and multivariate community structure for macroinvertebrates and amphibians were not affected by litter type at any point, though there was a weak trend for higher total community abundance in mesocosms with sedge litter relative to those with pine and maple litter (Figure 4). Species richness, however, was significantly affected by leaf litter type in the catch-and-release samples, with sedge litter ponds supporting more species on average than pine litter ponds (Figure 2). This effect was not seen in the census samples from August (Figure 3). Algae standing stocks, both periphyton and phytoplankton, were unaffected by litter types (Figure 9); but again, differences in growth rates of algae due to litter type could have gone undetected if the variation was short term or if more algal production resulted in more herbivory.

Leaf litter, as a source of nutrients and carbon, is expected to be important for the productivity of bacteria, fungi, and algae in aquatic systems (Tuchman et al., 2002; Gessner et al., 2007; Stoler and Relyea, 2011). Many aquatic taxa, including most macroinvertebrates and

amphibians, feed on these basal trophic groups, and therefore the productivity of higher trophic levels is expected to depend on leaf litter as well (Egglishaw, 1964; Yanoviak, 1999; Stoler and Relyea, 2011).

Leaf litter quality in aquatic systems is often defined by its rate of nutrient release: fast-decaying litter is considered higher quality than recalcitrant, slow-decaying litter (Webster and Benfield, 1986; Yanoviak, 1999). Some studies have shown that increases in productivity, induced by increasing leaf litter quality, can cause an increase in the diversity and abundance of macroinvertebrates in aquatic systems, often as a result of habitat choice or oviposition site selection (Srivastava and Lawson, 1998; Yanoviak, 1999; Richardson et al., 2004). Leaf litter quality has also been shown to affect the growth and survival of macroinvertebrates and amphibians in aquatic systems as well (Rubbo and Kiesecker, 2004; Williams et al., 2008; Stoler and Relyea, 2011). While our results show a significant difference in litter decomposition rate between the different leaf species, this variation in quality only affected the macroinvertebrate and amphibian community temporarily. However, the response was qualitatively what was predicted by the proposed relationship between diversity and productivity: ponds with higher-quality litter (sedge) supported more species than ponds with the lower-quality litter (pine). Similarly, we detected a weak trend for higher total community abundance in the higher-quality litter type as well. Therefore, leaf litter quality caused predictable, but short-term variation in biodiversity and community structure in our experiment.

It is unclear why local litter types would be important in the monthly catch-and-release samples, but not the August census samples. Moore et al. (2004) suggest that any study of detrital inputs should recognize that detritus is not a static resource: its quality can change over time. Temporal variation in nutrient release of the different litter types may therefore be

responsible for the time-dependency of the relationship between litter type and species richness. Detailed measurements of decomposition rates through time, as well as algal productivity through time, may have shown a temporal variation in nutrient release that would corroborate this hypothesis. Another possible explanation for the temporal variation in litter effects is that the strength of habitat selection at each site could have weakened over time as the higher-quality habitats became saturated with competitors or predators, causing patch quality to converge across our litter treatments (Fretwell and Lucas, 1986; Kraus and Vonesh, 2010). It is important to note that our sampling procedure differed in June and July, making direct comparisons across time difficult. In addition to the biological mechanisms proposed above, another factor that could be responsible for the lack of a litter effect in August is this difference in sampling procedure: perhaps the litter treatments affected the relative abundance of taxa, with more even species distributions in the higher-quality litter. This could cause us to detect a species richness effect in the catch-and-release samples that may disappear in August simply because the mesocosms were sampled more intensively. However, if this was the case, we would expect the multivariate analyses to show significant litter effects in July and August due to differences in relative abundance distributions—these effects were not detected.

Implications

It is clear that colonization is an important factor controlling community structure, but while it is important in most of the established metacommunity models, colonization is usually viewed as a constant or stochastic process (Leibold et al., 2004). Metacommunity models would be greatly improved by incorporating non-random habitat selection behavior (Binckley and Resetarits, 2007).

The relative importance of litter type and plant community in determining community structure among the mesocosms in our experiment was clear: the terrestrial matrix surrounding the ponds was more important than the local litter type in the pond itself. The plant community differences we observed seem to be driven, in large part, by colonization of more species in the mesocosms placed in open-canopy areas. Thus, large scale colonization dynamics seem to be important in this system. However, it is important to recognize that the evolution of this colonization behavior may have been driven by local factors within open-canopy ponds (such as increased productivity as a result of more light availability, higher temperatures, and higher litter), because canopy cover is consistently correlated with these factors (Binckley and Resetarits 2007; 2009). Litter type may be important to pond animals, but because other more obvious cues (such as canopy cover) are correlated with litter types, there is no selection pressure for organisms to be able to distinguish ponds based on the type of litter in the pond.

While not as strong as the plant community effects, leaf litter types did cause significant variation in community structure. We showed that species richness was lower in lower-quality litter types in the catch-and-release samples, with an interaction suggesting that this pattern is stronger in open-canopy areas. We cannot determine whether the litter effects in our experiment are the result of habitat selection or post-colonization processes. Perhaps habitat selection could cause the observed litter \times plant community interaction if taxa in open-canopy areas are more selective than those in forested areas. Alternatively, the litter \times plant community interaction may be explained by more post-colonization extinction in open-canopy areas based on litter quality. Our experimental manipulations may have caused an “ecological trap” (Shlaepfer, Runge, and Sherman, 2001) in the open-canopy areas, in which organisms chose to colonize ponds with low-quality litter in an area that usually has high-quality litter based on the surrounding plant

community. Litter types may have weaker effects in forested areas because the species in those areas are adapted to utilizing the lower-quality litter resources that characterize those plant communities.

There has been considerable interest in determining how changes (both natural and anthropogenic) in terrestrial plant communities may affect aquatic communities (Batzer and Mosner, 2000; Rubbo and Kiesecker, 2004; Skelly et al., 2005; Goodman et al., 2006; Leroy and Marks, 2006; Swan and Palmer, 2006; Going and Dudley, 2008; Williams et al., 2008; Earl et al., 2011; Stoler and Relyea, 2011). Our results suggest that processes that change litter types (species invasions, succession, etc.) may have weak, predictable effects on aquatic communities. However, other studies indicate that litter effects may prove to be difficult to foresee due to the often species-specific nature of litter chemistry and the unpredictable interactions between multiple litter types (Stoler and Relyea, 2011). Processes that open forest canopies (logging, fire, etc.), on the other hand, are predicted to consistently increase local diversity within ponds. Therefore, maintaining open-canopy ponds is likely critical to the preservation of biodiversity of pond systems. However, over 25% of the species in our study only occurred in closed-canopy, forested areas. While most species prefer open-canopy ponds, and more species coexist in these areas, it is clear that some species prefer closed-canopy ponds (Felix et al., 2010; Earl et al., 2011). Therefore, in order to maximize regional diversity, management strategies that preserve both closed- and open-canopy ponds are needed. It is also important to consider that distinct communities in open- and closed-canopy ponds results in less connectivity than may be expected, which has drastic effects on metacommunity dynamics (Binckley and Resetarits, 2009). Many species may only disperse among ponds within similar plant communities, resulting in less available habitat than one might predict based on the total number of ponds in an

area. It is critical to understand the factors that cause and maintain patterns of community structure if we are to advance predictive theory and create strategies for conservation, especially given the current decline of biodiversity worldwide (Butchart et al., 2010).

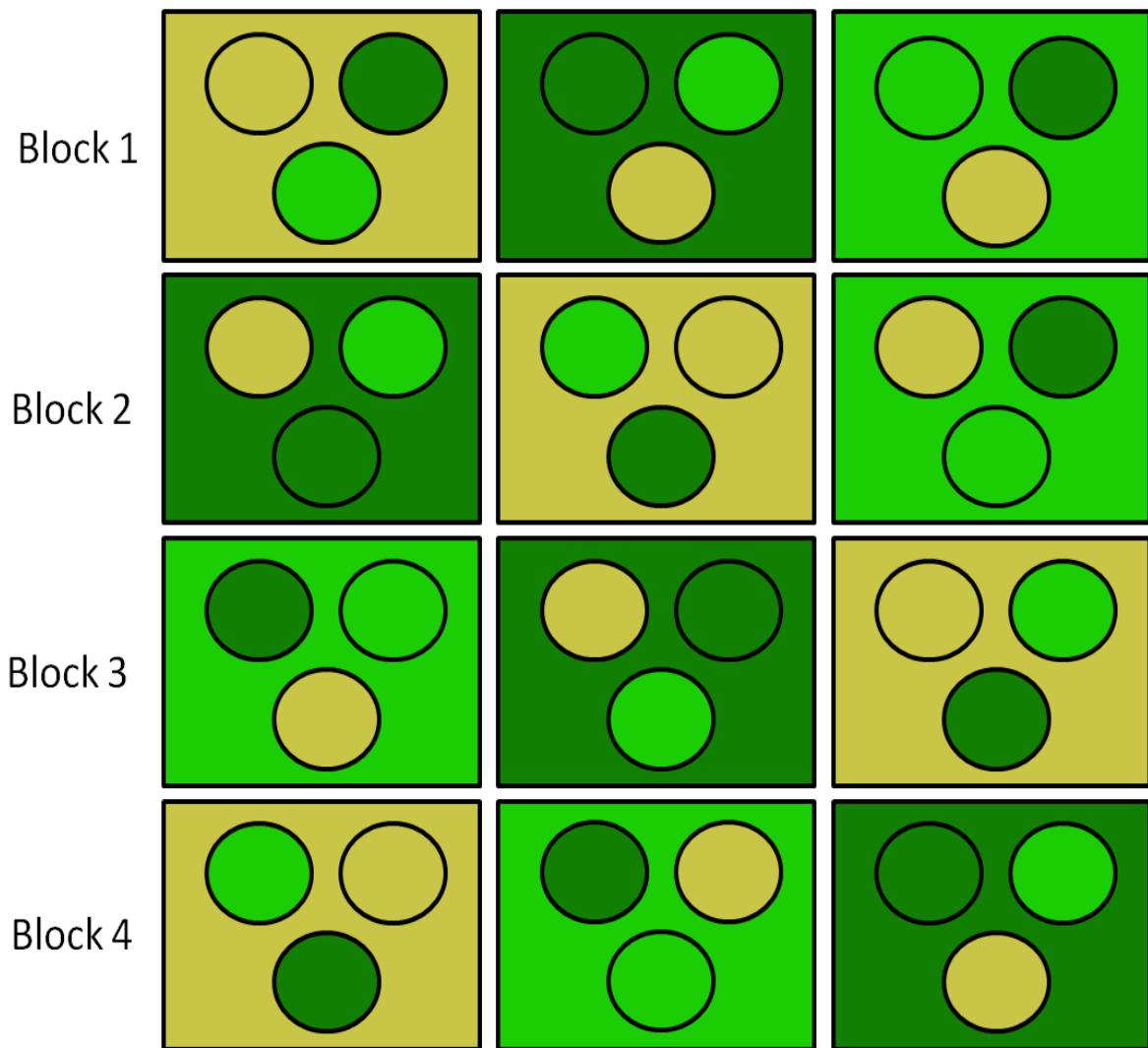


Figure 1: The split-plot experimental design. Rectangles represent independent sites in different plant communities (open-canopy: tan, pine forest: dark green, hardwood forest: light green). Circles represent the type of leaf litter in each mesocosm (sedge: tan, pine: dark green, maple: light green).

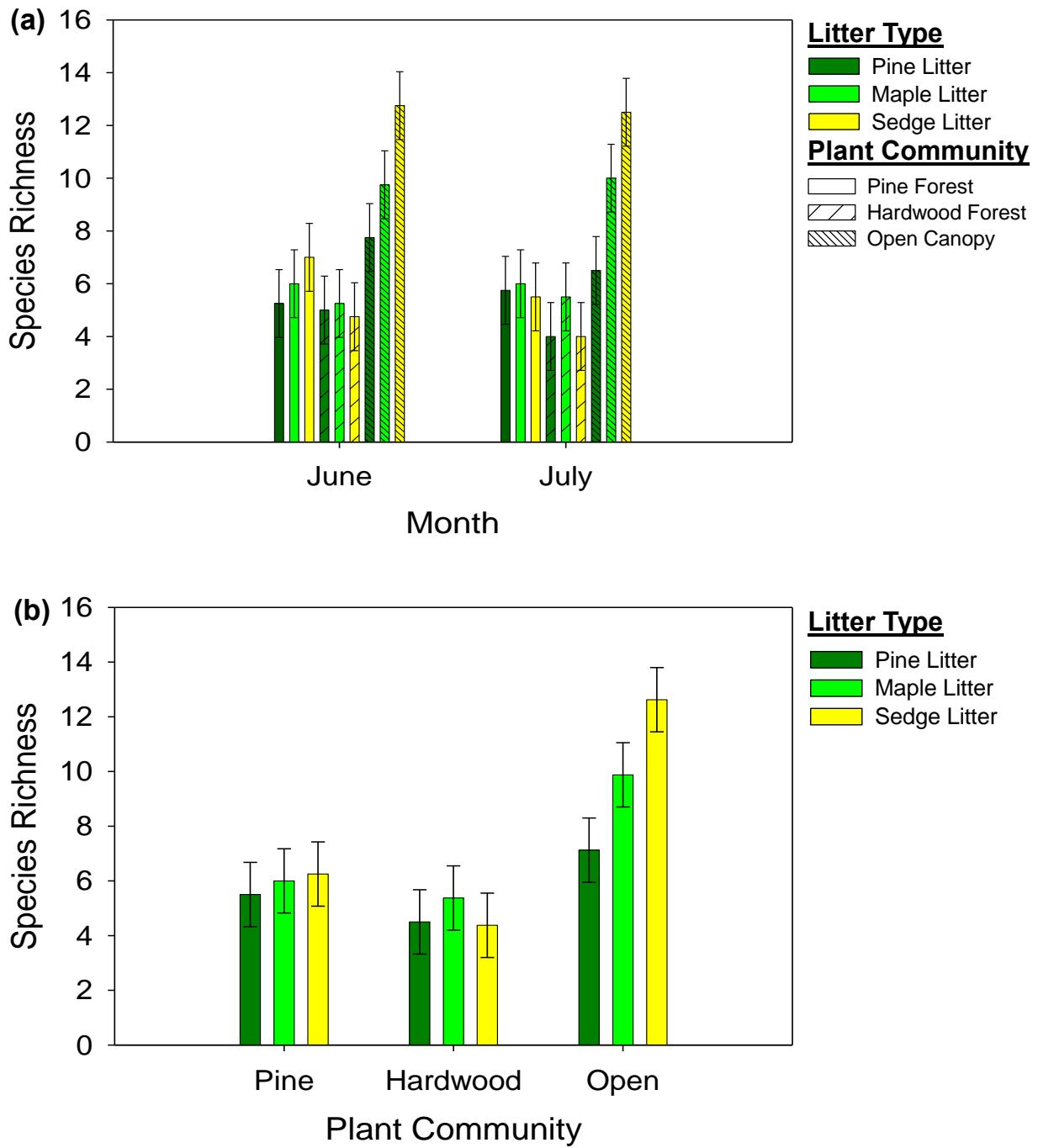


Figure 2: Species richness in the monthly catch-and-release samples. Panel (a) shows the average species richness ($LS\text{-mean} \pm SE$) for each treatment in June and July. Each bar is coded based on litter type (color) and plant community (superimposed lines). Panel (b) shows the species richness ($LS\text{-mean} \pm SE$) in each treatment averaged across both months to highlight significant effects of litter, plant community, and the litter \times plant community interaction.

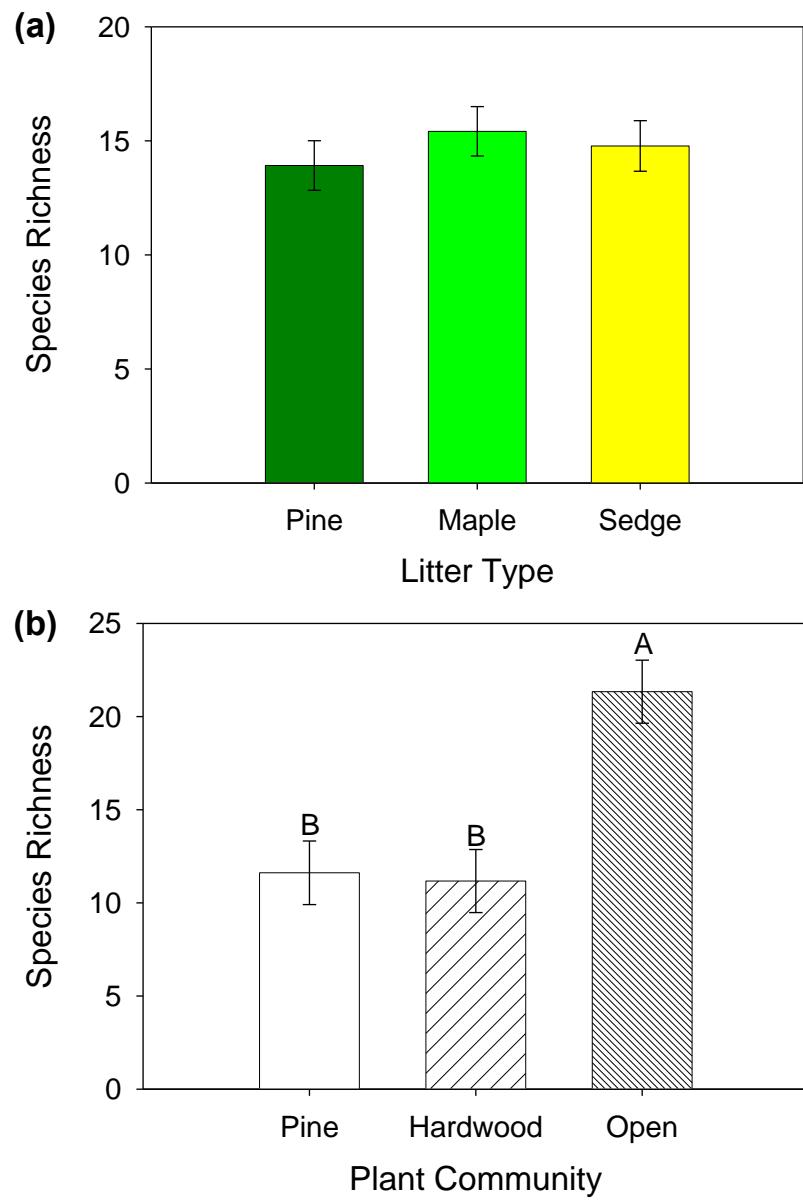


Figure 3: Species richness in August. Average species richness (LS-mean \pm SE) is shown for the different litter treatments (a) and plant community treatments (b). Letters above the bars indicate significantly different groupings based on Tukey's HSD.

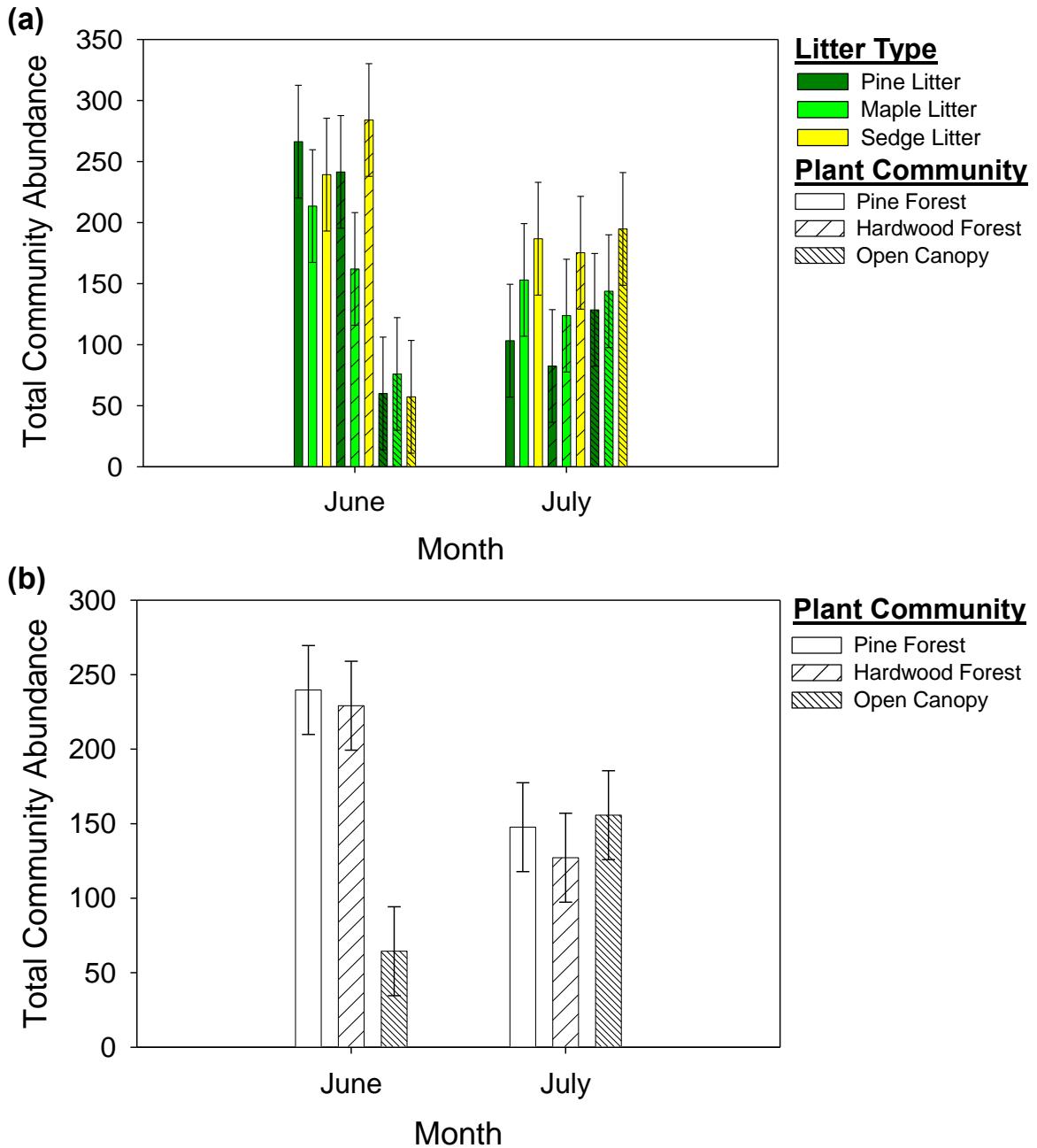


Figure 4: Total community abundance in the monthly catch-and-release samples. Panel (a) shows the average total community abundance (LS-mean \pm SE) for each treatment in June and July. Each bar is coded based on litter type (color) and plant community (superimposed lines). Panel (b) shows the total community abundance (LS-mean \pm SE) in each plant community (averaged across all litter types) in both months to highlight the month \times plant community interaction.

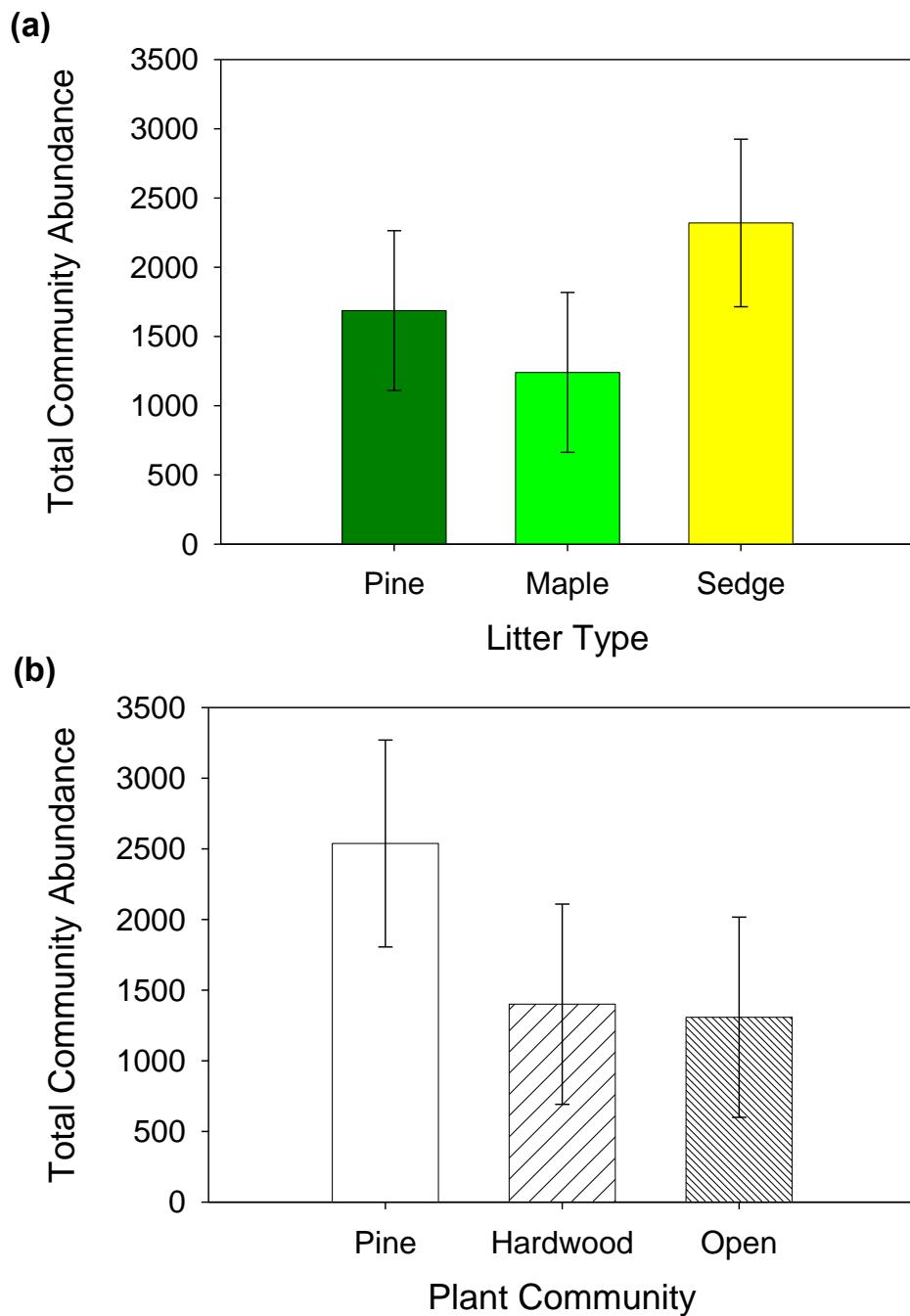


Figure 5: Total community abundance in August. Average total community abundance (LS-mean \pm SE) is shown for the different litter treatments (a) and plant community treatments (b).

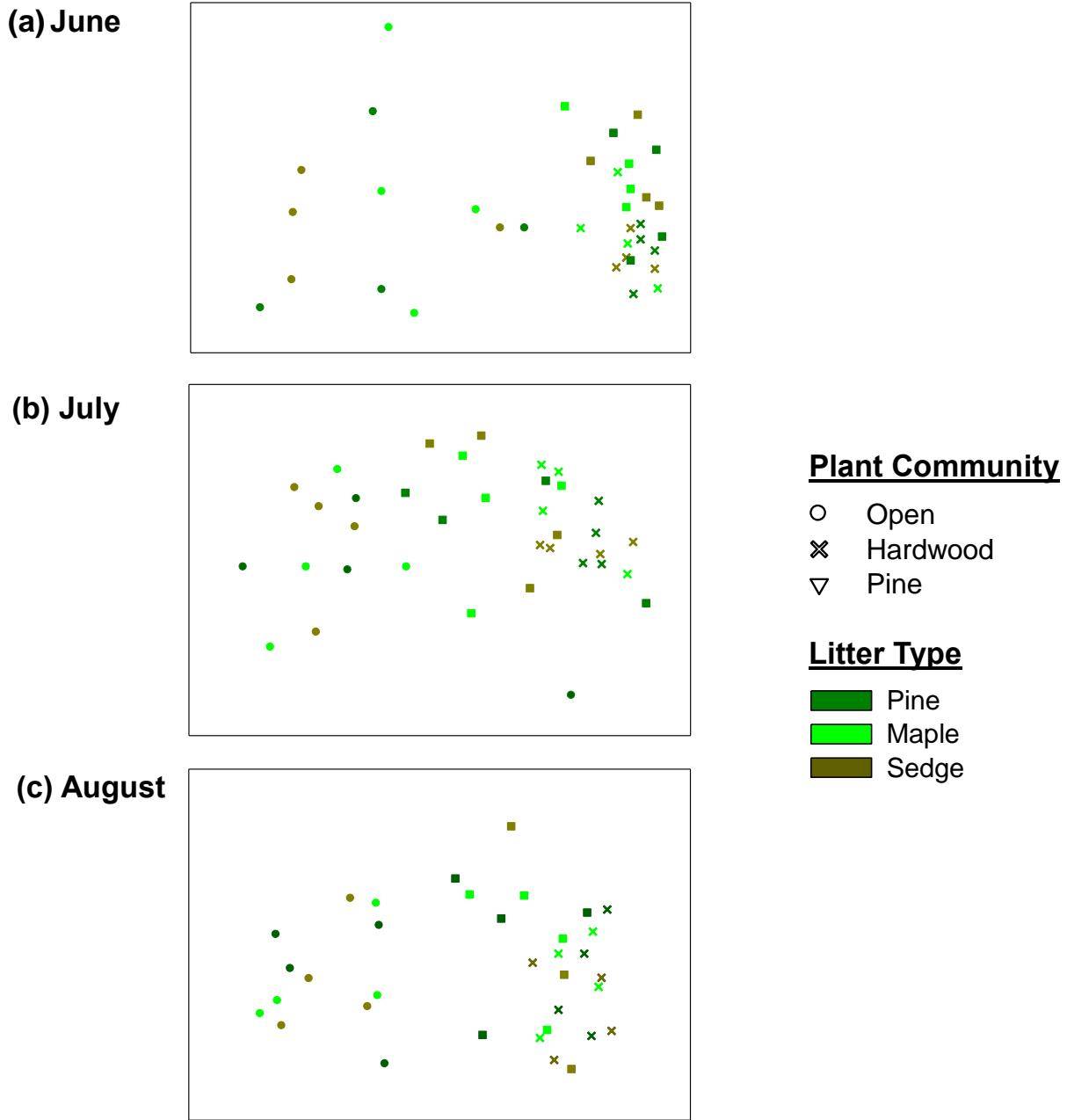


Figure 6: NMDS plots exploring litter and plant community effects simultaneously. Each experimental unit is coded based on its litter treatment (color), and its plant community treatment (symbol). Data from each month is shown in its own panel: (a) June, (b) July, and (c) August.

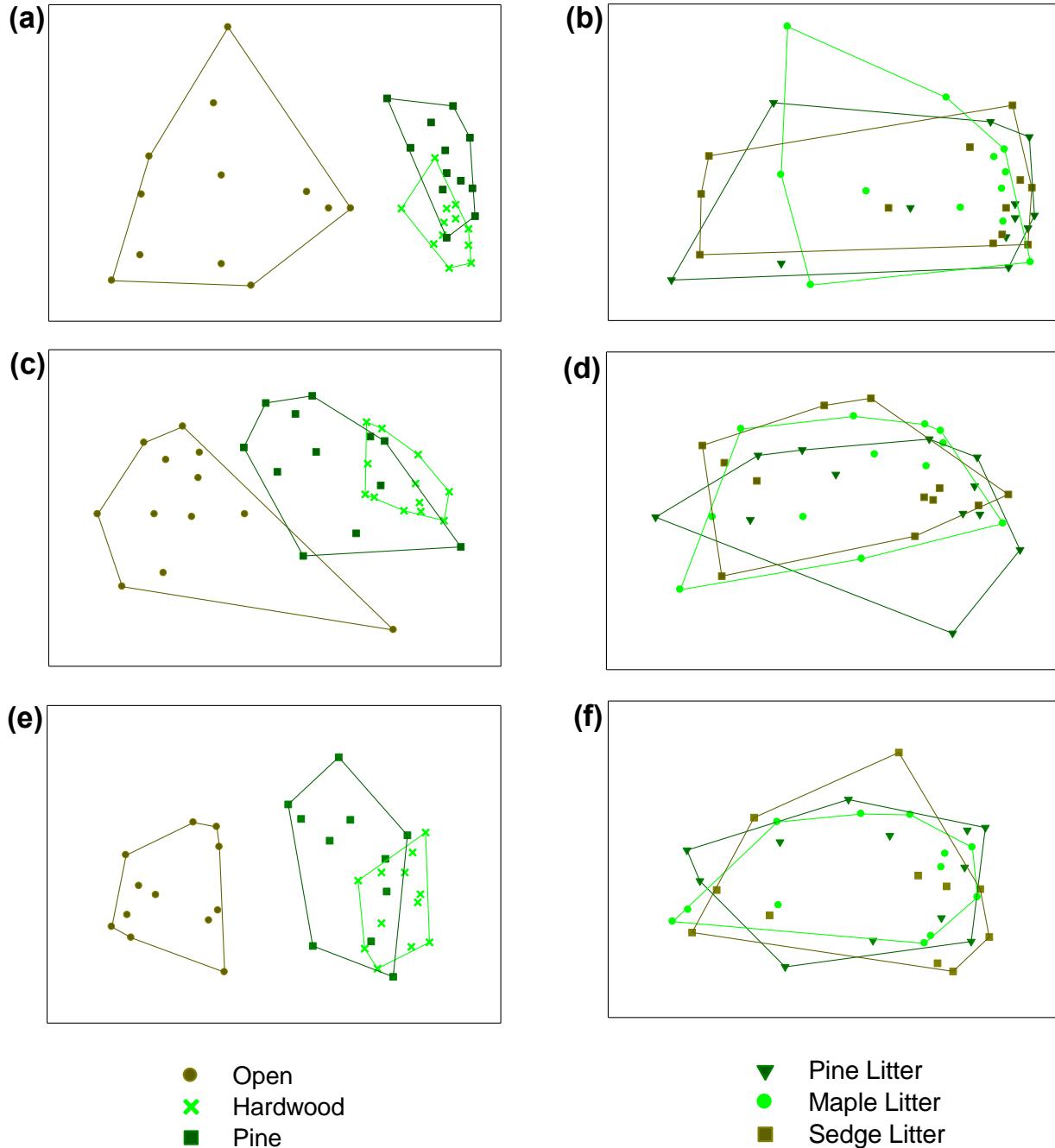


Figure 7: NMDS plots exploring litter and plant community effects independently. The left panels (a, c, and e) highlight the effect of plant community: the point representing each mesocosm is coded based on its plant community treatment. The right panels (b, d, and f) highlight the effect of litter type: the mesocosms are coded based on their litter treatments (see legends below figures). The points from each treatment are enclosed in the minimum convex hull of the same color. Data is shown for June (top, panels a and b), July (center, panels c and d), and August (bottom, panels d and e).

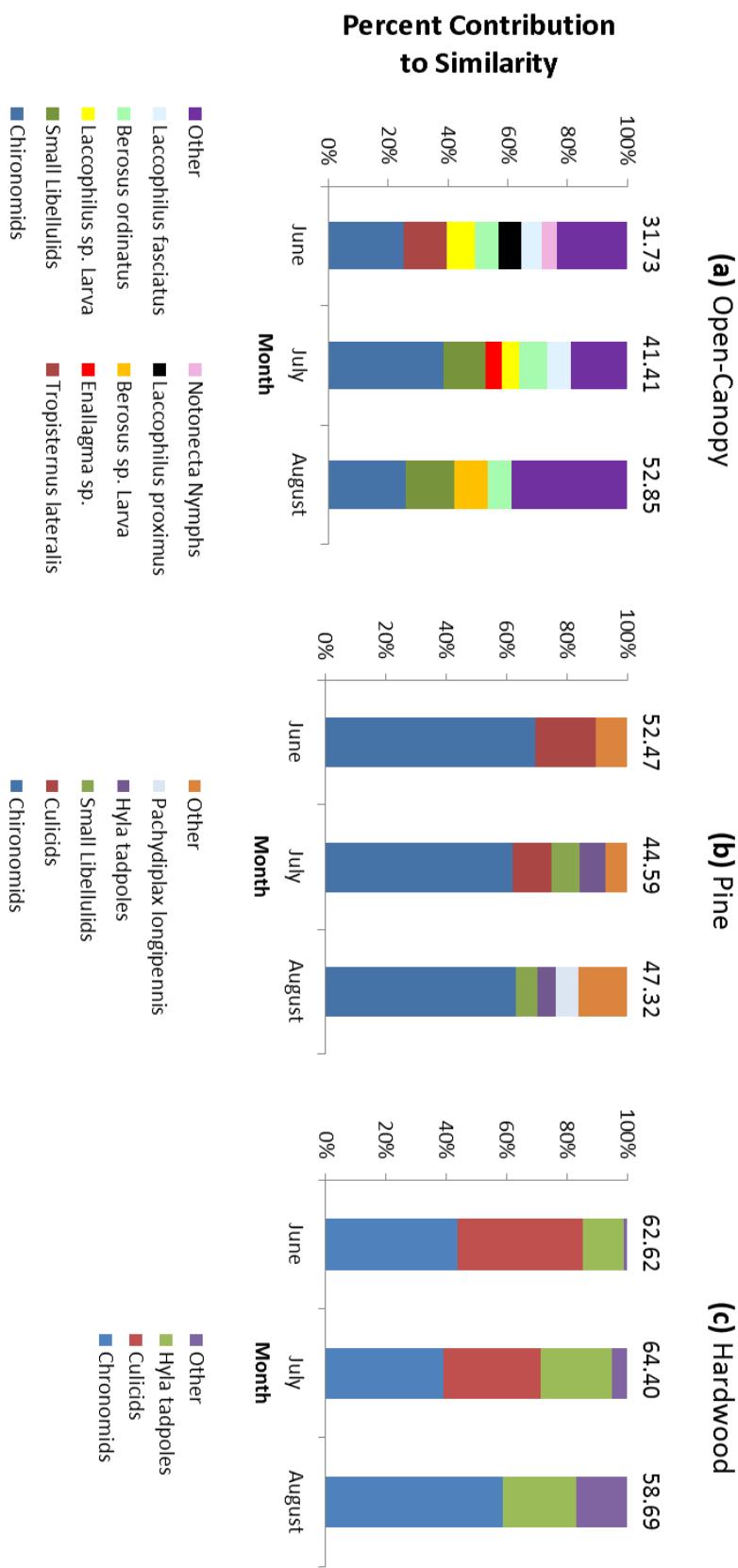


Figure 8: Percent contribution of taxa to similarity within each plant community: open-canopy (a), pine-dominated forest (b), and hardwood (c). The average similarity across plant community replicates is shown above the bar for each month. Taxa that contribute more than 5% to the overall similarity are grouped together as “other”.

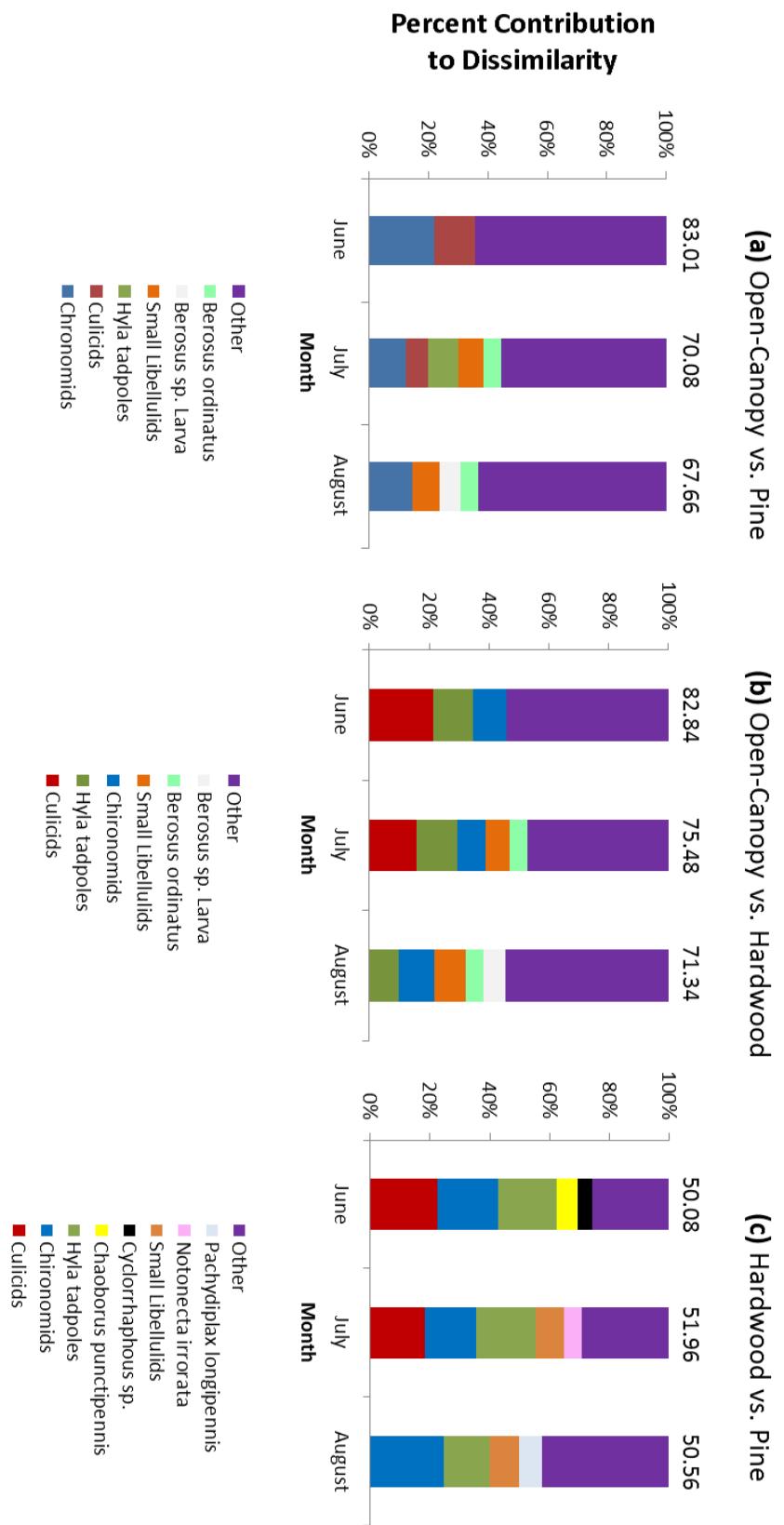


Figure 9: Percent contribution of taxa to dissimilarity among plant communities: open-canopy versus pine-dominated forest (a), open-canopy versus hardwood-dominated forest (b), and hardwood-dominated forest versus pine-dominated forest (c). The average dissimilarity among plant community treatments is shown above the bar for each month. Taxa that contribute more than 5% to the overall dissimilarity are shown. Taxa that account for less than 5% of the dissimilarity are grouped together as “other”.

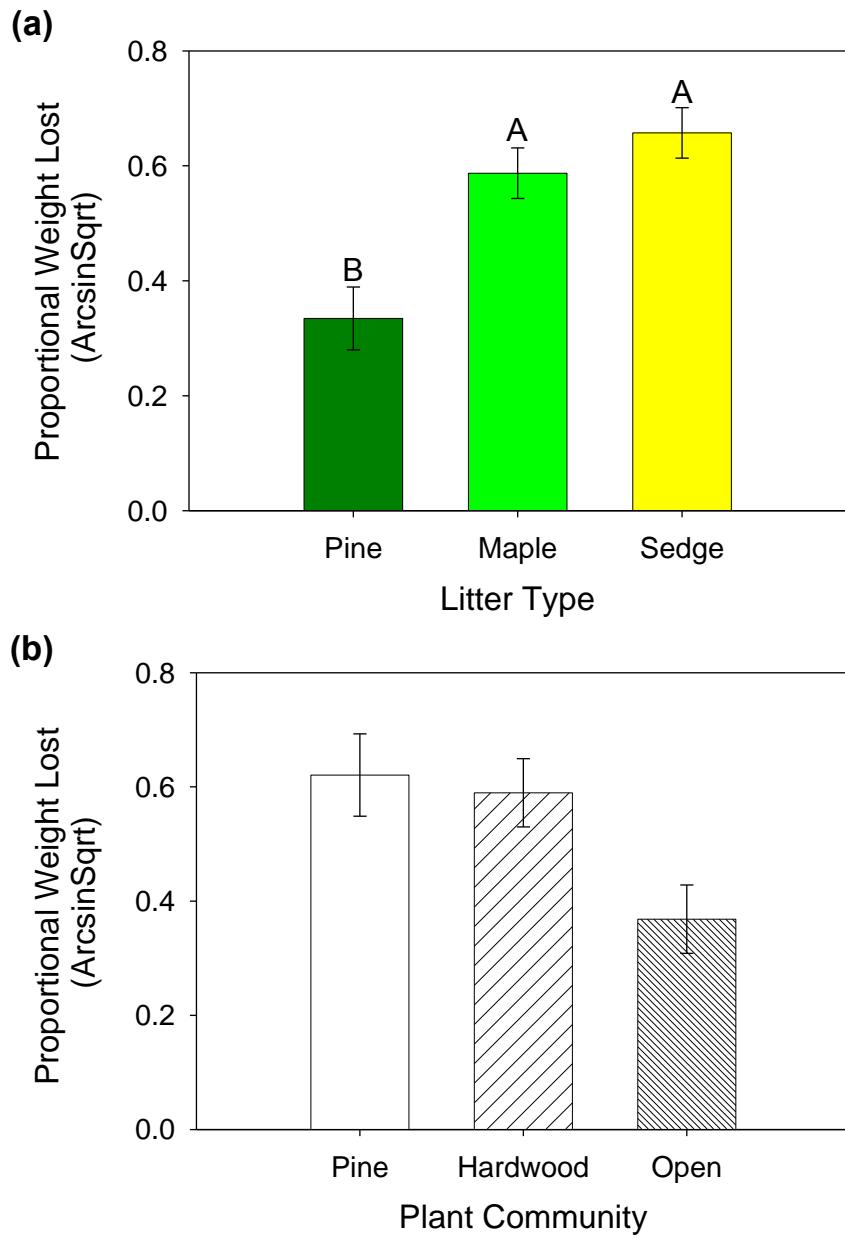


Figure 10: Leaf decomposition. Values represent the proportion of initial weight lost during the experiment after arcsine-square root transformation. Average proportional weight loss (LS-mean \pm SE) is shown for the different litter treatments (a) and plant community treatments (b). Letters above the bars indicate significantly different groupings based on Tukey's HSD.

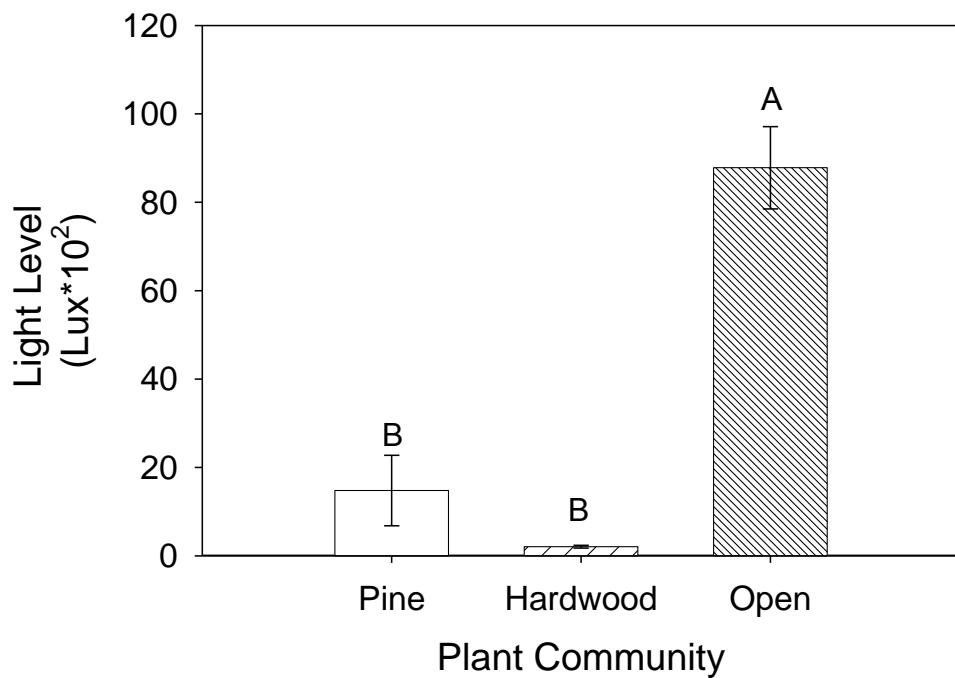


Figure 11: Light levels. Values represent the average light levels LS-mean \pm SE among the plant community treatments. Letters above the bars indicate significantly different groupings based on REGW post-hoc test.

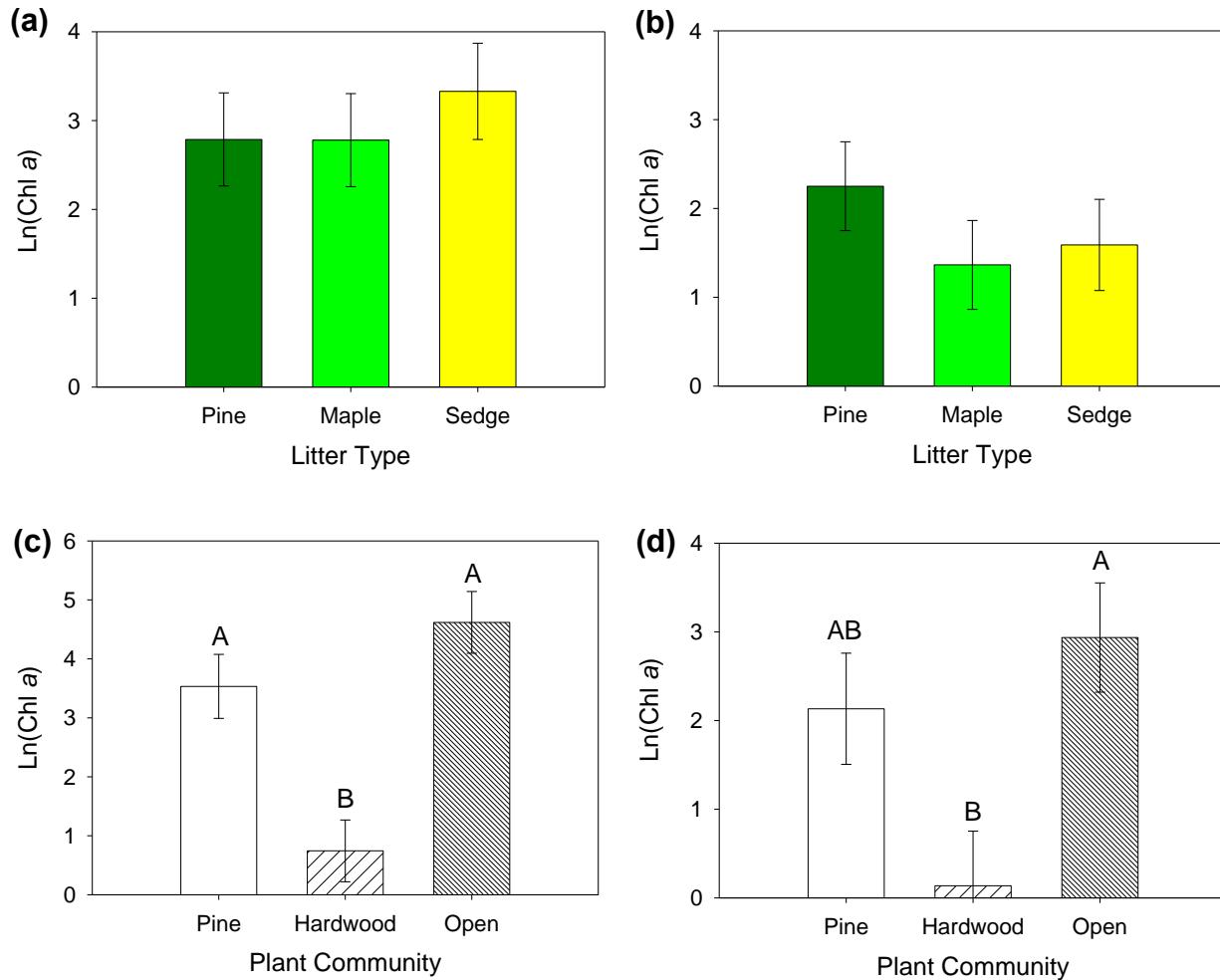


Figure 12: Periphyton and Phytoplankton standing stocks. Periphyton results are displayed on the left (panesl a and c), while phytoplankton results are shown on the right (panels b and d). Values represent chlorophyll *a* measurements after natural-log transformation. Average chl *a* (LS-mean \pm SE) is shown for the different litter treatments (top, a and b) and plant community treatments (bottom, c and d). Letters above the bars indicate significantly different groupings based on Tukey's HSD.

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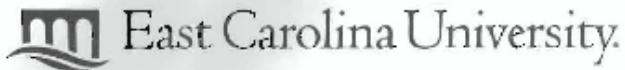
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APPENDIX: IACUC APPROVAL LETTER



Animal Care and
Use Committee

212 Old Engineering
Building, Greenville, NC
East Carolina University
Greenville, NC 27858

June 21, 2010

252 744-2436 office

252 744-2395 fax

David Chalcraft, Ph.D.
Department of Biology
Howell Science Complex
East Carolina University

Dear Dr. Chalcraft:

Your Animal Use Protocol entitled, "The Effects of Leaf Litter Type on Biodiversity and Community Structure of Temporary Ponds." (AUP #D244) was reviewed by this institution's Animal Care and Use Committee on 6/21/10. The following action was taken by the Committee:

"Approved as submitted"

A copy is enclosed for your laboratory files. Please be reminded that all animal procedures must be conducted as described in the approved Animal Use Protocol. Modifications of these procedures cannot be performed without prior approval of the ACUC. The Animal Welfare Act and Public Health Service Guidelines require the ACUC to suspend activities not in accordance with approved procedures and report such activities to the responsible University Official (Vice Chancellor for Health Sciences or Vice Chancellor for Academic Affairs) and appropriate federal Agencies.

Sincerely yours,

A handwritten signature in black ink, appearing to read "Robert G. Carroll, Ph.D."

Robert G. Carroll, Ph.D.
Chairman, Animal Care and Use Committee

RGC:jd

enclosure

