Project Title: Promoting and Demonstrating the Utility of Native Warm Season Grass Based Biofuels and Field Margins for Pollinators, Birds, and Carbon Sequestration

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Project Duration: 09/22/10 - 09/21/14 (including a 1-year no-cost extension)

Sponsor Award #: 69-3A75-10-158

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## Executive Summary

This project investigated how biofuel crop and harvest, grazing, and planting mixture influenced insect communities with an emphasis on pollinators, particularly bees. We also investigated a wide range of conservation practice treatments. While most previous research has focused on just a few aspects of conservation practices, our project provided comprehensive information about the effects of these practices on insect communities in agricultural landscapes. This project was conducted at three study sites: Prairie, Bryan Farms, and Scooba. Results from the Prairie site showed that an ungrazed mixture of native warm season grasses (NWSG planting) and Indiangrass monoculture could be more suitable for improving abundance of pollinators than other practices, especially during summer when pollinators are abundant. Results from the Bryan Farms site suggested that there was a relatively stronger effect of biofuel crop type (NWSG planting vs. switchgrass monoculture) on pollinators compared to harvest frequency (multiple vs. single harvest). Additionally, our findings from the Scooba site indicated that pine stand age was an important factor influencing the abundance of pollinators and all insects. Generally, there was a higher abundance of pollinators in 5-year-old pine stands with relatively open canopies compared to 10-year-old pine stands with mainly closed canopies. Results from the bloom-time survey part of our project offer baseline information that can be used for pollinator management. For example, establishing plant species (e.g., Illinois bundleflower, Tick-seed sunflower, and Partridge pea) that bloom for long periods of time or establishing a combination of plant species (e.g., Butterfly milkweed and Black-eyed Susan) that bloom at different times of year may support pollinators for extended periods of time and increase pollination services. While more study is needed to fully understand the processes driving how insect communities and pollinating bees respond to differing agricultural practices, here we have provided valuable information regarding how conservation practices influence insect communities in agricultural landscapes.

## Introduction

Invertebrates are key components in agricultural ecosystems where they function in positive (e.g., pollinators, nutrient cycling, and wildlife food sources) and negative (e.g., crop pests) ways. Insect pollination is essential for maintaining plant diversity within natural ecosystems with two-thirds of all flowering plants being dependent on insect pollinators (Kearns and Inouye, 1997). However, land-use changes, habitat fragmentation, and agricultural practices threaten many pollinators and other important arthropods. Likewise, row crop monocultures can negatively impact pollinating insects by not providing the diversity of plants necessary to sustain diverse pollinator communities (Cane and Tepedino 2001). Moreover, conversion of native plant communities to exotic species may increase non-native pollinators while decreasing locally adapted native species. Pollinating insects and other beneficial arthropods play an essential role in the function and integrity of agroecosystems and land-use planning and agricultural management regimes should consider impacts on the arthropod community.

Biofuel crop production, which has been expanded greatly in recent years (Fargione et al. 2009), could potentially fragment landscapes and increase monoculture planting. Currently, the effects of many of these biofuel crops on floral-visiting insects and beneficial arthropods are largely unknown. Gardiner et al. (2010) conducted a study of pollinating bees within three types of biofuel crops (switchgrass, prairie, and corn). Overall, they found higher abundances and species richness of bees within the switchgrass and prairie compared to corn and this supports the idea that biofuels with greater plant diversity support higher abundance and species richness of pollinators. Other arthropods will likely show varying responses to biofuel crops (Landis and Werling 2010). For improved agricultural management practices involving biofuel production, land resource managers and biofuel growers need better information on consequences of various biofuel practices.

Conservation buffers and herbaceous field margins have been shown to provide important habitat in agricultural landscapes for beneficial insects including pollinators and arthropods that prey upon agricultural pests (Marshall and Moonen 2002). Predatory arthropods are important in controlling populations of herbivorous insects in crops and may be more abundant at field margins adjacent to diverse natural herbaceous vegetation (Lang et al. 1999). Field margin habitats provide important overwintering site for arthropods and can provide habitat and food sources during times when fields barren (Pfiffner and Luka 2000, Bäckman and Tiainen 2002). Beneficial insects in conservation buffers can serve as a source for the field when populations dwindle within crop fields after crop management activities (Sorenson and Outward 1999). In fields where pesticides are heavily used, buffers may serve as refugia allowing these insects to recolonize fields early, when pest populations are still low (Alomar et al. 2002). Various studies have demonstrated that predatory beneficials in borderless fields are less abundant and have fewer predator-days (days present in the field) by which to control pest insects (Alomar et al. 2002). Increasing the abundance and diversity of flowering plants in field margins has been shown to increase the number and diversity of pollinating insects (Bäckman and Tiainen 2002, Marshall and Moonen 2002, Mänd et al. 2002). When the ecology of major crop pests is considered and pest and beneficial insect populations are monitored, field borders can enhance natural controls of pest populations without causing economic loss to crops.

The following objectives were met by the integrative approach of this project:

- **Demonstrated the effectiveness of native warm season grass** (NWSG) plantings as habitat for **diverse pollinator communities** in managed systems. Practices used for demonstration included (not exhaustive): (1) herbaceous conservation buffers practices periodically disturbed by burning or disking, (**upland habitat buffers** implemented under CRP- CP33, and the related Practice Codes 332, 386, 393, and 412 implemented under EQIP); and (2) **block-type plantings**--Practice Codes 327,645 & 647 implemented under EQIP and CRP – CP-2, CP10, CP25).

The above objective was used to accomplish the following objectives:

- Established demonstration plantings of NRCS-recommended pollinator habitat seed/plant mixes and determined if these mixes were providing the expected pollinator habitat while also performing the intended conservation function;
- Documented regional time of bloom of native plants and non-invasive, non-native plants and monitored the specific pollinators foraging upon these plants;
- Documented the benefits to other wildlife species (mainly birds) of improving pollinator habitat (this objective was met through the matching dollars);
- Developed regional, crop-specific guidance specifying the vegetative species, landforms, and necessary acreage to support appropriate populations of managed and wild pollinators per unit area (e.g., acres) of pollinated crops (i.e., described the components of the landscape).

- Developed region-specific “recipes” of pollinator-friendly plant species to fulfill specific pollinator needs in both natural and agricultural situations;
- Developed strategies to integrate pollinator habitat management into the agricultural working lands matrix to promote holistic, ecosystem-based conservation plans that supported the full suite of ecosystem services;
- Developed guidelines and management strategies for establishing and maintaining the foraging and breeding needs for specific pollinators and other beneficial insects;
- Developed, revised, and refined NRCS practice standards as they related to provision of pollinator habitat.

Because this project was married to several other NWSG practices and biofuel plantings, it addressed foci in other sub-categories including ***Energy*** and ***Climate Change Mitigation and Adaptation***. This provided a unique opportunity for a systems approach to documenting the effects of these practices on myriad ecosystem services. Briefly, the other projects included ***11 different types of biofuel and native grass management practices*** across a wide range of contexts including agricultural and forestry landscapes. These practices included varying biofuel harvest and forage techniques, intercropping with woody plants, and the use of NWSG for cattle forage.

- Grazed Bermudagrass Pasture*:* Bermudagrass pastures are the traditional standard for summer forages (Practice code #528).
- Grazed Native Warm Season Pasture*:* A mix of big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), and Indian grass [(*Sorghastrum nutans*); Practice code #528 &550].
- Ungrazed Native Warm Season Pasture*:* Same species were planted as above except without grazing (Practice code #550).
- Grazed Indiangrass*:* A monotypic pasture of Indian grass [(*Sorghastrum nutans*); Practice code #528 & 550).
- Switchgrass monoculture with a single biomass harvest in the fall.
- Switchgrass monoculture with multiple biomass harvests to simulate haying (Practice code #511).
- NWSG mix with a single biomass harvest in the fall: A mix of big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium soparium*), Indian grass (*Sorghastrum nutans*), and selected prairie forbs.
- NWSG mix (same as directly above) with multiple harvests to simulate haying (Practice code 511).
- Woody planting (Pine species) similar to CP-3 plantings.
- Woody planting (Pine species) with woody biomass harvest.
- Woody planting (Pine species) with intercropped switchgrass planting similar to silvopasture (Practice code 381) and alley cropping.

The effectiveness of these practices to provide habitat for birds and carbon sequestration were the major goals of these projects. The ***additional*** documentation and demonstration of the pollinator communities’ responses to these practices was most beneficial.

Review of Methods

1. Study site, study design, and practice

The project area encompassed 3 counties in Mississippi that included two unique agro-ecosystems: the Black Prairie region and Interior Flatwoods and Central Hills (Fig. 1). These areas also included three Congressional districts in Mississippi. These regions represented a gradient in agriculture and forestry intensity. In the Black Prairie region, the landscape was a mix of row crops (41%), mostly soybeans and corn, with a measurable amount of pastureland (18.86%). The average farm size in this region was 244 acres. The operators in this region were predominantly white (15,655) while African American operators were the second highest with 1,630 operators. In the Flatwoods and Central Hills region, the predominant agriculture was timber production (44%), livestock production (20.81%), and small fields of soybeans and corn (28.39%). The average farm was 196 acres. White Americans operated the majority of the farms (16,145) and African Americans operated 2,287 farms.

Sixteen different types of practices associated with biofuel production, native grass planting, and cattle grazing were established across these regions. At each region, one large study unit (or site) was selected to implement practices and 12-16 plots (pastures, fields, or stands) were established at each site.

<u>Black Prairie region</u>

*Mississippi State Prairie Research Unit (Prairie site)*

Prairie site was used to establish practices associated with diverse and monotypic NWSG plantings, Bermuda grass, and Indian grass (See below and Fig. 2). Unlike most other studies that have been based on small-plot research, the large-scale design (twelve 20-25 acre paddocks totaling 274 acres) was implemented on this site. At Prairie site, three blocks of areas were selected (Fig. 2). Each block was composed of 4 pastures, resulting in a total of 12 pastures. Within a block, each of following 4 practices were randomly assigned to each pasture.

- Grazed Bermudagrass Pasture*:* Bermudagrass pastures are the traditional standard for summer forages (Practice code #528).
- Grazed Native Warm Season Grass (NWGS) Pasture*:* A mix of big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), and Indian grass [(*Sorghastrum nutans*); Practice code #528 &550].
- Ungrazed Native Warm Grass (NWGS) Pasture*:* Same species planted as above except without grazing (Practice code #550).
- Grazed Indiangrass*:* A monotypic pasture of Indian grass [(*Sorghastrum nutans*); Practice code #528 & 550).

<u>Interior Flatwoods and Central Hills region</u>

*Bryan Farms (Bryan Farms site)*

Bryan Farms was an EQIP eligible producer in Clay County, Mississippi. Across this 5400 acre farm, approximately 25% of the land base was allocated to a myriad of conservation practices under a diversity of Conservation Programs including EQIP, WHIP, and CRP. Bryan Farms site included one more block (a total of 16 fields, 18-20 acres in size except 2 fields that are 12-13 acres in size) than Prairie site, but most blocks were closely located (Fig. 3). Within a block, each of following practices were randomly applied to each field that was a soybean field previously.

- Switchgrass monoculture with a single biomass harvest in the fall.
- Switchgrass monoculture with multiple biomass harvests to simulate haying (Practice code #511).
- NWSG mix with a single biomass harvest in the fall: A mix of big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium soparium*), Indian grass (*Sorghastrum nutans*), and selected prairie forbs.
- NWSG mix (same as directly above) with multiple harvests to simulate haying (Practice code #511).

In 2011, no harvest was performed to ensure establishment of grasses. The first cut (single biomass harvest) was conducted at all fields in mid-April 2012 and the second cut was applied to 8 fields assigned for multiple biomass harvests in late-June 2012. However, establishment of switchgrasses at two fields (Switch M in block B and Switch S in block C, Fig. 3) was poor and thus those fields were excluded for analysis.

*The Scooba Unit (Scooba site) of Weyerhauser Company's forest holdings*
Scooba site, although located on commercial forestland, was established and managed in ways that frequently establish the pattern for forest management regimes on private lands in the Midsouth. Two different aged woody plantings (pine species) with intercropped biofuels plantings of switchgrass were established on this site (See below list and Fig. 4). At Scooba site, three large 5 years old pine stands were selected and 2 plots (each plot size = 23-25 acres) were established within a stand. One plot was used to represent pine stand without intercropped switchgrass (PI) and the other represented a pine stand with intercropped switchgrass (SI). In addition to these stands, six 10-year-old pine stands were chosen: three stands received switchgrass intercropping treatment (T-PISI) and the other three stands had no treatment (T-PISI). One plot (23-27 acres) was established at each of those six stands. Switchgrasses were planted between late-May and early-June 2012 and harvested during December 2013.

- Young (5 years old) pine planting similar to CP-3 plantings: PI.
- Young (5 years old) pine planting with intercropped switchgrass planting similar to silvopasture (Practice code 381) and alley cropping: SI.
- Relatively old (10 years old) pine planting similar to CP-3 plantings: T-PI.
- Relatively old (10 years old) pine planting with intercropped switchgrass planting similar to silvopasture (Practice code #381) and alley cropping: T-PISI.

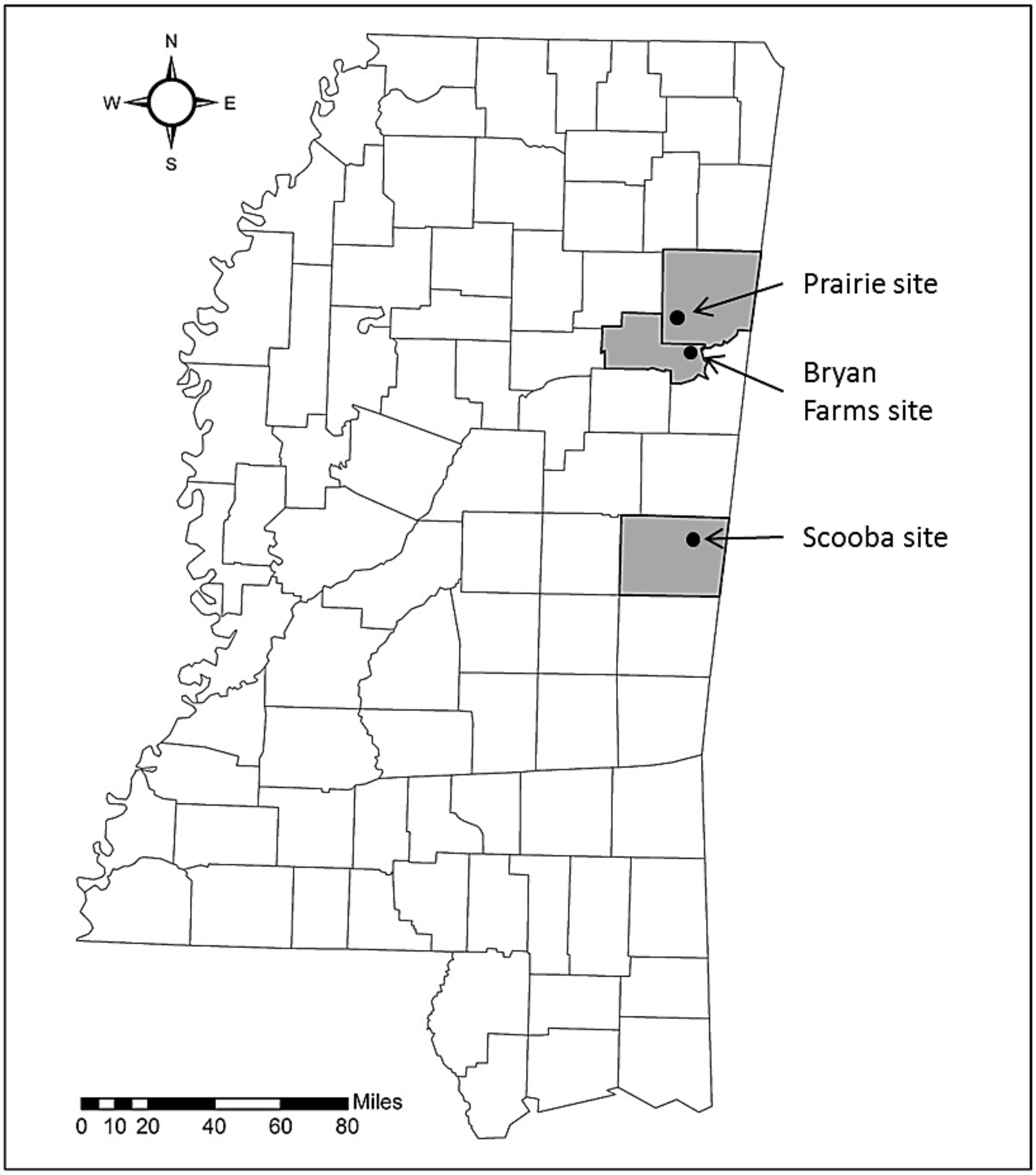



Figure 1. Location of study sites: Prairie, Bryan Farms, and Scooba site in Monroe, Clay, and Kemper County, respectively.

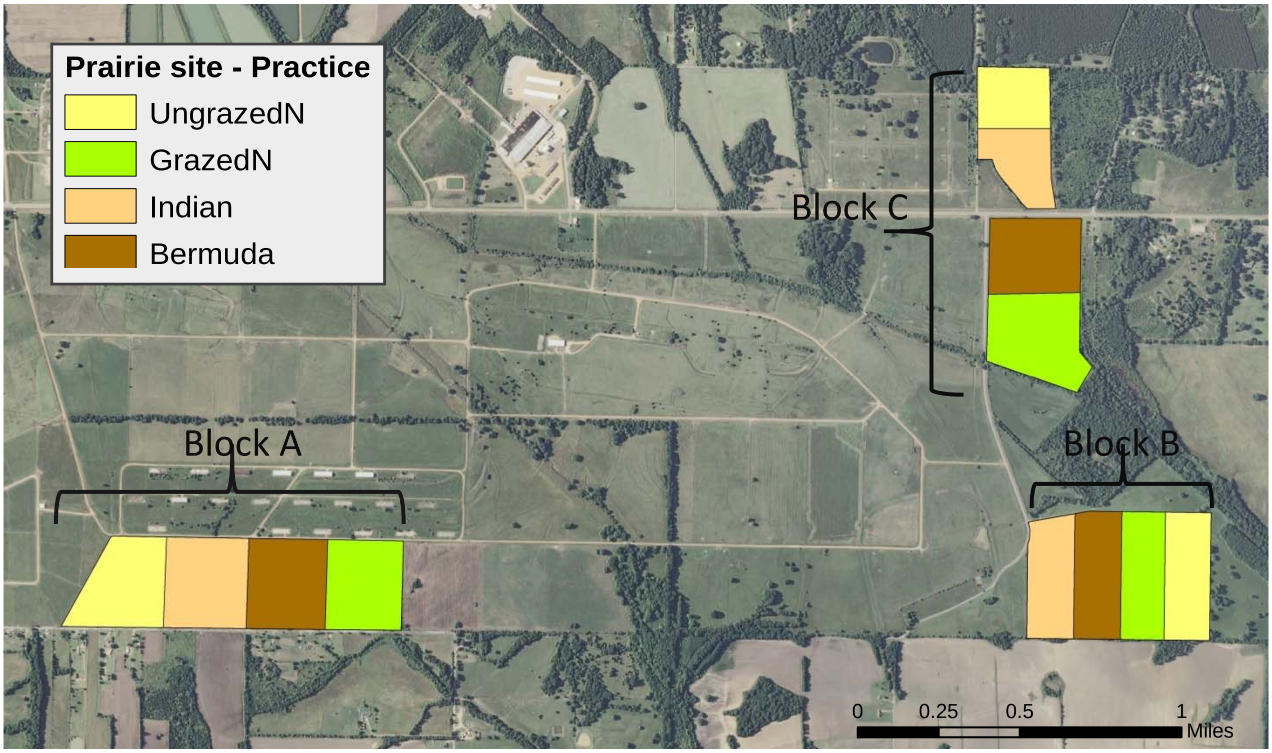



Figure 2. Sampling design at Prairie site. Practice: Bermuda, Grazed Bermudagrass pasture (Practice code # 528); GrazedN, Grazed native warm season pasture (Practice code # 528 & 550); Indian, Grazed Indiangrass, (Practice code # 528 & 550); UngrazedN, Ungrazed native warm season pasture (Practice code # 550).

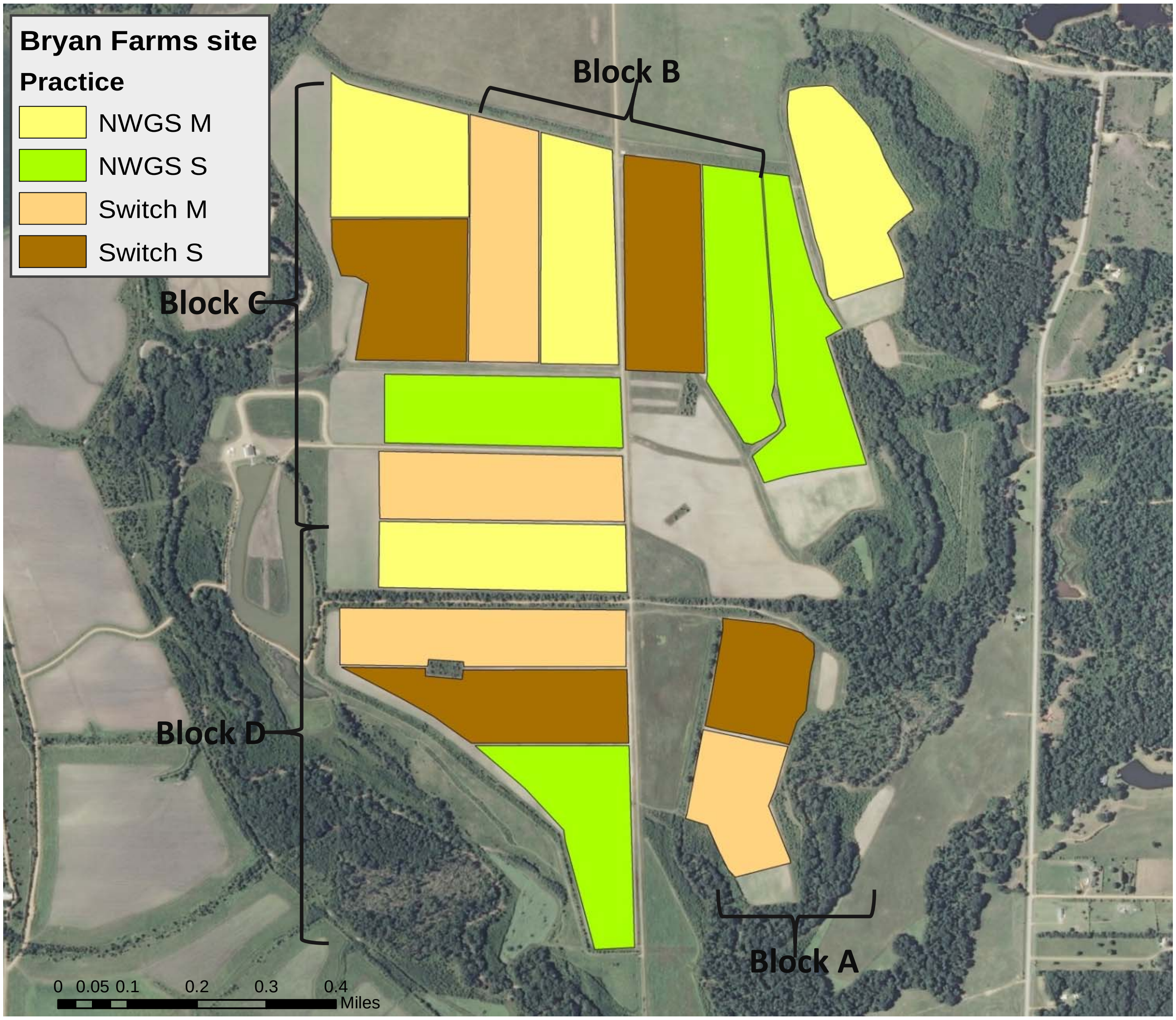



Figure 3. Sampling design at Bryan Farms site. Practice: NWSG M, NWSG mix with multiple biomass harvests (Practice code # 511); NWSG S, NWSG mix with a single biomass harvest; Switch M, Switchgrass monoculture with multiple biomass harvests (Practice code # 511); Switch S, Switchgrass monoculture with a single biomass harvest.

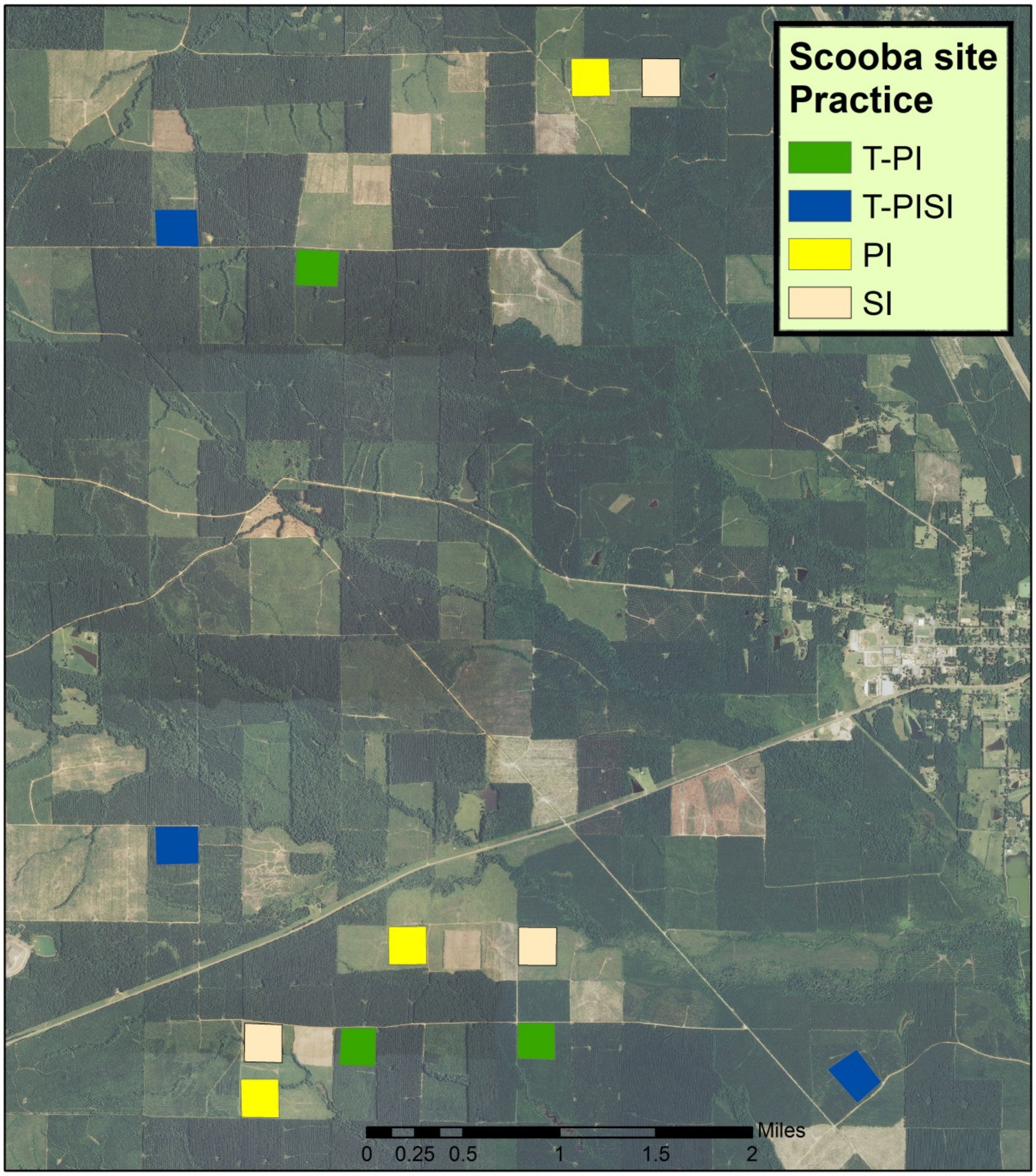



Figure 4. Sampling design at Scooba site. Practice: T-PI, 10-year-old control stand similar to CP3 planting; T-PISI, 10-year-old stand with intercropped switchgrass; PI, 5-year-old control stand similar to CP3 planting; SI, 5-year-old stand with intercropped switchgrass.

2. Insect collection

For insect (pollinators and other insects) sampling, colored pan traps were used because they were known to be successful at capturing a wide diversity of floral visiting insects and can allow for a more complete analysis of the insect floral visiting community within our biofuel treatments (Campbell and Hanula 2007). Three different colored (blue, white, and yellow) 12-oz bowls were prepared as one set of pan traps and those bowls were filled with a soap-water solution (See Appendix A for detail preparations). One set of bowls was placed to the height of flowers or vegetation if forbs had not begun to flower at three sample locations within a plot (pasture, field, or stand). Pan trap sets were spaced > 25 m from the edge of a plot to avoid edge effects and > 50 m from the nearest pan trap set to minimize dependency between pan trap sets. At Prairie site, where 75% of the fields were grazed by cattle, cattle panel was installed surrounding traps to exclude cattle from the traps.

To account for seasonal variation in insect abundance, trapping was performed each month during May-October (or November) 2011-2012 at Prairie site and Bryan Farms site (Table 1) and during May-August 2013-2014 at Scooba site (Table 2). Traps at all sites were set up at each location once or twice (about 10-14 days apart) a month except in June (Scooba site) or August (Prairie site and Bryan Farms site). Contents of each trap were collected three days following trap set up. The number of traps collected at each study site was slightly lower than the number of traps set up due to weather issues. Collected insect samples were preserved in a 70% ethanol solution (See Appendix A for detail process of trap collection) and sent to an entomologist (Josh Campbell) for identification.

3. Bloom-time survey

During summer (June-August), a weekly bloom-time survey was conducted at Prairie site in 2012 and Bryan Farms site in 2012 and twice a month at Brayan Farms site in 2013. A bloom-time survey was also conducted during other seasons (spring, May; fall, September-October), but once in May and twice per month during fall. A total of 20 visits were made at Prairie site and a total of 19 visits and 10 visits were made at Bryan Farms site in 2012 and 2013, respectively. For a boom-time survey, a random staring point was chosen at the edge of plot (pasture or field) and one observer walked at a reasonably brisk pace in a straight line (transect) through the center of the plot to the opposite edge. A list of target flowing species was provided to the observer before survey (See Appendix B). The list was derived from forb species intentionally planted in NWSG and CP33 buffers combined with species typically not planted but frequently present and of value to pollinators. Among those species in the list, Partridge pea, Illinois bundleflower, Tick-seed sunflower, Black-eyed Susan, Maximillian's sunflower, Butterfly milkweed, Purple coneflower, Grey-headed coneflower, Showy ticktrefoil, Rounded lespedeza, and Purple prairie clover were considered as major blooming plants (See Appendix B for scientific name). The observer circled species observed in bloom (i.e., visible flower) while walking through the plot. The observer also recorded other species not on the list. All surveys were conducted between 6-11 am because some flowers may close during the heat of the day. No surveys were performed during measurable precipitation events.

4. Data analyses

All insects were counted and identified to family level and all bees (5 families: Andrenidae, Apidae, Colletidae, Halictidae, and Megachiliidae) to genus level. Insects were grouped into 4 guilds in accordance with their foraging strategy: predators/parasites/parasitoids, pollinators, herbivores (phytophagous insects), and others (fungivores, omnivores, etc.). Bees were also grouped into two nest placement guilds: ground nesting bees and non-ground nesting bees (wood/stem/cavity nesting and eusocial hive).

Although both insects and bees were considered in all analyses, our statistical analyses were focused on bee abundance and community structure because of their importance as pollinators. To examine the effect of practices on bees, abundance (number of individuals) of total bees (Total), ground nesting bees (Ground), non-ground nesting bees (Non-ground), and bees belonging to Family Apidae (Apidae) were used as response variables. These variables were log(x+1)-transformed prior to analysis to avoid biases because of too high or low abundance of some genera at a sampling location (trap). Homogeneity of variance assumption was examined using Levene's test. Heterogeneous variance structure was dealt with by allowing different variances among treatments ("varIdent" function) in the model (Zuur et al. 2009). Due to variation in abundance among months, all analyses were performed separately by month. A general linear mixed model was used with practice as a fixed effect and pasture (Prairie site), field (Bryan Farms site) or stand (Scooba site) as random effects. All analyses were performed in R, using package "nlme."

The association of bee community composition at genus level with practice was examined using analysis of similarity (ANOSIM; R package, vegan) and redundancy analysis (RDA). At each study site, genera detected at 3-4 traps (< 10% of total number of traps) were excluded for this analysis to avoid biases due to the occurrence of uncommon genera. We used ANOSIM to test whether there was a significant compositional difference between groups by comparing distances between groups with distances within groups. Bray-Curtis similarity was used as a measure of distance. The value of the ANOSIM statistic R ranges from 1 to -1: R value from 0 to +1, increasing dissimilarity among groups; R value=0, no relationship; R value from 0 to -1, increasing similarity among groups (more than within a group). The RDA is one of canonical ordination methods commonly used for the analysis of community composition data. It is a combination of regression analysis and principal component analysis (Borcard et al. 2011).

Abundance was also calculated for all insects and each of the foraging strategy guilds. In addition, richness of insect family (family richness) and of bee genus (genus richness) was considered to investigate seasonal variations in insect and bee diversity and effect of practices on their diversity. The relationship between practices and structure of insect community (foraging strategy guilds) was investigated by calculating relative frequency (or proportion) of 4 foraging strategy guilds which was based on mean abundance of each guild at each practice.

Table 1. Number of traps (a set of three colored bowls) collected at Prairie site and Bryan Farms site during 2011-2012. No traps were set up at Prairie site in May and November 2011. Practice at Prairie site: Bermuda, Grazed Bermudagrass pasture (Practice code # 528); GrazedN, Grazed native warm season pasture (Practice code # 528 & 550); Indian, Grazed Indiangrass, (Practice code # 528 & 550); UngrazedN, Ungrazed native warm season pasture (Practice code # 550). Practice at Bryan Farms site: NWSG M, NWSG mix with multiple biomass harvests (Practice code # 511); NWSG S, NWSG mix with a single biomass harvest; Switch M, Switchgrass monoculture with multiple biomass harvests (Practice code # 511); Switch S, Switchgrass monoculture with a single biomass harvest.

| **Study Site** | **Practice** | **2011** | | | | | | | | | **2012** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | May[b] | Jun[a] | Jul | Aug[b] | Sep[a,b] | Oct | Nov[b] | **TOTAL** | | May | Jun[b] | Jul | Aug[c] | Sep[b] | Oct[b] | **TOTAL** |
| Prairie | UngrazedN | – | 6 | 18 | 18 | 9 | 18 | – | 69 | | 18 | 17 | 18 | 25 | 18 | 17 | 182 |
| | GrazedN | – | 11 | 15 | 15 | 9 | 18 | – | 71 | | 18 | 18 | 18 | 27 | 18 | 17 | 187 |
| | Indian | – | 6 | 17 | 12 | 9 | 18 | – | 68 | | 18 | 18 | 18 | 27 | 18 | 18 | 185 |
| | Bermuda | – | 7 | 19 | 11 | 9 | 18 | – | 71 | | 18 | 18 | 18 | 27 | 18 | 18 | 188 |
| | **TOTAL** | – | 30 | 69 | 56 | 36 | 72 | – | 279 | | 72 | 71 | 72 | 106 | 72 | 70 | 463 |
| | | | | | | | | | | | | | | | | | |
| Bryan Farms | NWSG M | 11 | 24 | 23 | 12 | 11 | 24 | 12 | 117 | | 21 | 12 | 22 | 36 | 9 | 12 | 112 |
| | NWGS S | 12 | 23 | 23 | 12 | 12 | 22 | 12 | 116 | | 20 | 12 | 24 | 36 | 9 | 12 | 113 |
| | Switch M | 11 | 24 | 24 | 11 | 11 | 23 | 12 | 116 | | 22 | 11 | 24 | 36 | 9 | 12 | 114 |
| | Switch S | 11 | 22 | 23 | 12 | 12 | 22 | 12 | 114 | | 24 | 12 | 22 | 33 | 9 | 12 | 112 |
| | **TOTAL** | 45 | 93 | 93 | 47 | 46 | 91 | 48 | 463 | | 87 | 47 | 92 | 141 | 36 | 48 | 451 |

[a] Only one trapping occasion at Prairie site
[b] Only one trapping occasion at Bryan Farms site
[c] Three trapping occasions at both sites

Table 2. Number of traps (a set of three colored bowls) collected at Scooba site during 2013-2014. Practice: T-PI, 10-year-old control stand similar to CP3 planting; T-PISI, 10-year-old stand with intercropped switchgrass; PI, 5-year-old control stand similar to CP3 planting; SI, 5-year-old stand with intercropped switchgrass.

| **Practice** | **2013** | | | | | | **2014** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | May[a] | Jun | Jul | Aug | **Total** | | May[a] | Jun[b] | Jul | Aug | **Total** |
| Old (T-PI) | 9 | 17 | 17 | 18 | 61 | | 9 | 27 | 17 | 18 | 71 |
| Old+Switch (T-PISI) | 9 | 18 | 16 | 18 | 61 | | 9 | 27 | 17 | 18 | 71 |
| Young (PI) | 9 | 18 | 18 | 18 | 63 | | 9 | 27 | 18 | 18 | 72 |
| Young+Switch (SI) | 9 | 18 | 16 | 18 | 61 | | 8 | 28 | 18 | 17 | 71 |
| **TOTAL** | 36 | 71 | 67 | 72 | 246 | | 35 | 109 | 70 | 71 | 285 |

[a] One sampling occasion
[b] Three sampling occasions

Findings

# 1. Prairie Site

## 1.1. Bees

### 1.1.1. General pattern

A total of 5454 bees of 23 genera were captured during 2011-2012 (Table 3 and Table 4). Lasioglossum was the most abundant, comprising 80% of total bees in 2011 and 69% in 2012. Abundance of bees belonging to Apidae was 8.6% in 2011 and increased in 2011, accounting for 20.8% of total bees. However, this significant change might be influenced by more trappings in 2012 than 2011. There were variations in bee abundance between months. Abundance of most bee genera peaked during early-mid summer, particularly in June (2012) and July (2011) and then decreased throughout late-summer and fall (Table 4 and Fig.5-8).

### 1.1.2. Effect of practice

*Abundance*

In 2011, mean abundance per location was similar between practices although four locations in August showed higher mean abundance of total bees and ground nesting bees than any other locations (Fig. 5-8). However, more differences in mean abundance of total bees and ground nesting bees among practices were found in 2012, particularly in June (Fig. 5-6). Abundance of those bees was high at Indiangrass (I) and ungrazed native warm season pasture (N).

The results of generalized mixed modeling showed similar patterns. In 2011, practice did not significantly influence abundance of total bees and any of the nesting guilds (Table 5). Also, Apidae did not show significant response to practice. Compared to 2011, more cases of significant practice effect were observed in 2012. Abundance of total bees and of ground nesting bees was significantly influenced by practice in June.

*Genus richness*

Monthly genus richness and overall genus richness was greater in 2012 than in 2011 except September (Fig. 9). Effect of practice on genus richness varied by year. While grazed native warm season pastures showed relatively greater genus richness in 2011, genus richness was greater at Bermudagrass pastures in 2012. However, it should be pointed out that abundance of 50% of bee genera in 2012 and 33% in 2011 was very low, only 1-3 individuals captured. Those individuals were mostly trapped at grazed native warm season pasture and Indiangrass pasture in 2011 and at Bermudagrass pasture and ungrazed native warm season pasture in 2012, indicating genus richness could be biased by those low abundant genera.

*Composition of bee community*

At family level, Apidae was abundant evenly at all practices in 2011. Halicidae and Megachilidae were more abundant at ungrazed native warm season pasture (UngrazedN). However, these patterns changed in 2012: Apidae was abundant at UngrazedN, whereas Megachilidae was more often observed at Indiangrass pasture (Indian; Fig. 10).

At genus level, the results of ANOSIM showed bee community was dissimilar among four practices compared to within each practice in 2011 (ANOSIM statistic $R = 0.12$, $p = 0.03$) and 2012 (ANOSIM statistic $R = 0.12$, $p = 0.02$). However, low value of R indicates that the dissimilarity in bee community among practices is weak.

These patterns were also congruent with the results of RDA, which showed associations between practices and several genera but overall low explanatory power of practice: < 20% of variation in the data was explained by practices. Lasioglossum (L) was positively related to UngrazedN and Augochlorella (A3), Melissodes (M2), and Halictus (H) tended to respond positively to UngrazedN in 2011 (Fig. 11). A3 and H were also positively associated with Indian and Grazed native warm season pasture (GrazedN), respectively. Apis (A1) showed weak positive response to Bermuda. Other genera were not associated with practices. While most genera did not show clear responses to practice in 2012, several genera showed the similar pattern observed in 2011. UngrazedN had positive effect on L and M2. L and A3 showed positive responses to Indian.

1.2. Insects

1.2.1. General pattern

Over 44,800 insects from 25 families were trapped during 2011-2012 (Table 6 and Table 7); however, 82% of those insects were captured in 2012. Dolichopodidae was the most dominant both years, comprising 55% of total insects in 2011 and 73% in 2012. Abundance of each family showed seasonal variations. Unlike the pattern found in bee genus, total insect abundance peaked during fall in 2011 and in May in 2012. However, this pattern was driven by two insect families (Dolichiopodiae and Syphidae) in Order Diptera.

1.2.2. Effect of practice

*Abundance*

Like abundance of bee genus, there were more variations in mean abundance among locations than among practices and mean abundance per location were similar between practices (Fig. 12-15). However, mean abundance of total insects and of predator/parasite/parasitoid insects tended to be high at ungrazed native warm season pasture in June in 2011 but low in September in 2011 and June in 2012 (Fig. 12-13). Mean abundance of herbivore insects was relatively low at Bermudagrass pasture in Jun and July in 2011 and in August and September in

2012 (Fig. 14). Mean abundance of pollinators was slightly higher at ungrazed native warm season pasture during June-August in 2011 and June and October in 2012 (Fig. 15).

*Family richness*

Family richness of insects varied by month and showed different patterns between two years although overall family richness at each year was the same (Fig. 16). Family richness was greater in June in 2011 and decreased during fall, whereas family richness in 2012 tended to increase from summer to fall. Differences in family richness among practices were not as large as in bee richness. Family richness was slightly high at GrazedN in 2011 and Bermuda in 2012; however, this may be biased by very low abundant families that were mainly observed at GrazedN in 2011 and at Bermuda in 2012.

*Composition of insect community*

Among four foraging guilds of insects, predator/parasite/parasitoid was a dominant guild in all practices (Fig. 17). However, compared to other practices, insect community at UngrazedN was also more composed of pollinators, particularly in 2011. In addition, the proportion of herbivores was relatively higher at UngrazedN than at other practices.

Table 3. Family, genus (and species), and guild classification (based on nest placement) of bees detected during 2011-2012, at Prairie site, Mississippi.

| Family | Genus | Species | Nest placement guild |
|---|---|---|---|
| Andrenidae | Andrena | | Ground nesting |
| | | *Andrena macra* | |
| | Perdita | | Ground nesting |
| | | *Perdita octomaculata* | |
| | | 1Unknown *Perdida* spp. | |
| Apidae[a] | Apis | | Eusocial hive[b] |
| | | *Apis mellifera* | |
| | Bombus | | Ground nesting |
| | | *Bombus griseocollis* | |
| | | *Bombus pennsylvanicus* | |
| | | *Bombus bimaculatus* | |
| | | *Bombus impatiens* | |
| | Ceratina | | Wood/Stem nesting |
| | | *Ceratina calcarata* | |
| | | *Ceratina dupla* | |
| | | *Ceratina strenua* | |
| | Melissodes | | Ground nesting |
| | | *Melissodes bimaculata* | |
| | | *Melissodes boltoniae* | |
| | | *Melissodes tepaneca* | |
| | | *Melissodes comptoides* | |
| | | *Melissodes wheeleri* | |

| | | | |
|---|---|---|---|
| | | 2Unknown Melissodes spp.[c] | |
| | Melitoma | | Ground nesting |
| | | *Melitoma taurea* | |
| | Svastra | | Ground nesting |
| | | *Svastra atripes* | |
| | | *Svastra obliqua* | |
| | Xylocopa | | Wood nesting |
| | | *Xylocopa virginica* | |
| Colletidae | Hylaeus | | Stem nesting |
| | | *Hylaeus mesillae* | |
| | | *Hylaeus affinis* | |
| | | 1Unknown *Hylaeus* spp. | |
| Halictidae | Agapostemon | | Ground nesting |
| | | *Agapostemon virescens* | |
| | Augochlora | | Wood nesting |
| | Augochlorella | | Ground nesting |
| | Augochloropsis | | Ground nesting |
| | Nomia | | Ground nesting |
| | | *Nomia melanderi* | |
| | Dieunomia | | Ground nesting |
| | | *Dieunomia nevadensis* | |
| | Halictus | | Ground nesting |
| | | *Halictus poeyi* | |
| | Lasioglossum[d] | | Ground nesting |

| | | | |
|---|---|---|---|
| | Sphecodes | | Ground nesting (Cleptoparasite) |
| Megachilidae | Heriades | | Wood/Stem nesting |
| | Megachile | | Wood/Stem nesting |
| | | Megachile sculpturalis | |
| | | *Megachile sculpturalis* | |
| | | *Megachile campanulae* | |
| | | 1Unknown *Megachile* spp. | |
| | Osmia | | Wood/Stem nesting |
| | | *Osmia georgica* | |

[a] 1Unknown genus
[b] Considered as non- ground nesting for analysis.
[c] One of the species could be *M. communis* or *M. triodis*.
[d] *L. bruneri, L. callidum, L. mitchelli, L. pruninosum*, and other unknown Lasioglossum species.

Table 4. Number of bees trapped at Prairie site, Mississippi during 2011-2012. Note that more trapping occasions were performed in 2012.

| **Genus** | **2011** | | | | | | **2012** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Jun[1] | Jul | Aug | Sep[1] | Oct | **TOTAL** | May | Jun | Jul | Aug[2] | Sep | Oct | **TOTAL** |
| Lasioglossum | 284 | 1176 | 248 | 169 | 76 | 1953 | 262 | 849 | 529 | 260 | 166 | 44 | 2110 |
| Halictus | 36 | 26 | 4 | 9 | 2 | 77 | 7 | 54 | 19 | 3 | 5 | 3 | 91 |
| Augochlorella | 16 | 80 | 19 | 7 | 22 | 144 | 13 | 79 | 24 | 19 | 4 | 2 | 141 |
| Augochlora | 1 | 1 | 0 | 2 | 1 | 5 | 0 | 17 | 14 | 3 | 0 | 2 | 36 |
| Agapostemon | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Augochloropsis | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 3 | 4 | 2 | 0 | 1 | 10 |
| Nomia | 0 | 0 | 2 | 1 | 2 | 5 | 1 | 0 | 0 | 1 | 0 | 0 | 2 |
| Sphecodes | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Dieunomia | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Heriades | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Megachile | 5 | 1 | 0 | 2 | 3 | 11 | 1 | 4 | 2 | 0 | 0 | 1 | 8 |
| Osmia | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Andrena | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Perdita | 0 | 0 | 3 | 0 | 1 | 4 | 0 | 3 | 0 | 0 | 0 | 0 | 3 |
| Melissodes | 13 | 48 | 16 | 20 | 16 | 113 | 3 | 52 | 192 | 72 | 13 | 7 | 339 |
| Melitoma | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Svastra | 0 | 2 | 16 | 0 | 0 | 18 | 0 | 1 | 60 | 53 | 3 | 0 | 117 |
| Bombus | 0 | 5 | 0 | 1 | 0 | 6 | 3 | 5 | 5 | 14 | 3 | 1 | 31 |
| Ceratina | 0 | 8 | 2 | 0 | 0 | 10 | 1 | 18 | 3 | 20 | 4 | 1 | 47 |
| Xylocopa | 2 | 0 | 0 | 0 | 0 | 2 | 1 | 1 | 0 | 1 | 0 | 0 | 3 |
| Apis | 2 | 3 | 3 | 2 | 48 | 58 | 39 | 28 | 7 | 5 | 4 | 11 | 94 |
| Unknown Apidae | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hylaeus | 0 | 0 | 1 | 0 | 1 | 2 | 2 | 0 | 0 | 1 | 0 | 0 | 3 |
| **TOTAL** | 360 | 1350 | 315 | 215 | 172 | 2412 | 336 | 1115 | 859 | 456 | 202 | 74 | 3042 |

[1]only one trapping occasion
[2] three trapping occasions

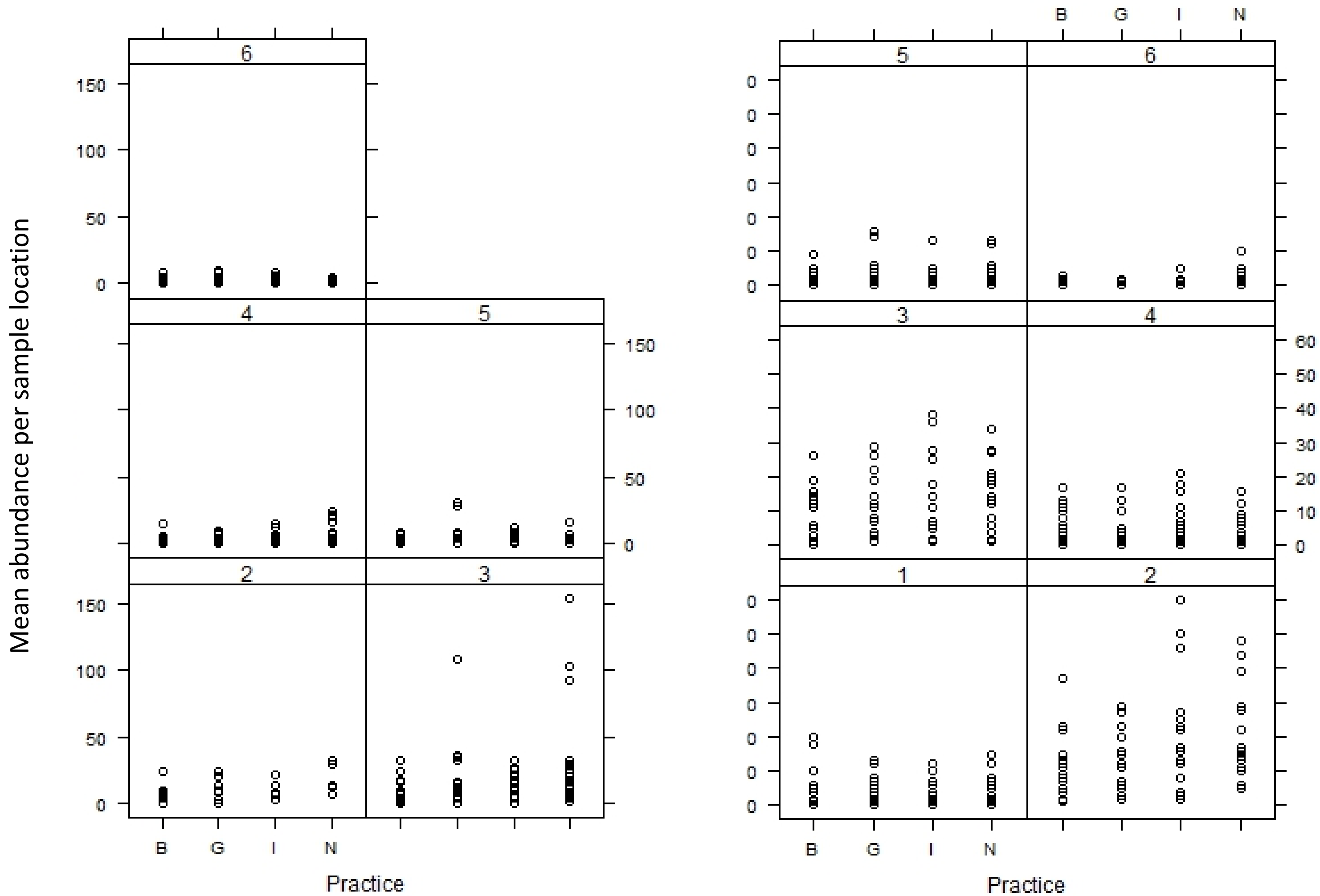



Figure 5. Mean abundance of all bees captured at each location (a set of traps, represented as “sample location” in the graph) during May (denoted as 1), June(2), July(3), August(4), September(5) and October(6) in 2011 (Left) and 2012 (Right). Practice: B, Grazed Bermudagrass pasture (Practice code # 528); G, Grazed native warm season pasture (Practice code # 528 & 550); I, Grazed Indiangrass, (Practice code # 528 & 550); N, Ungrazed native warm season pasture (Practice code # 550).

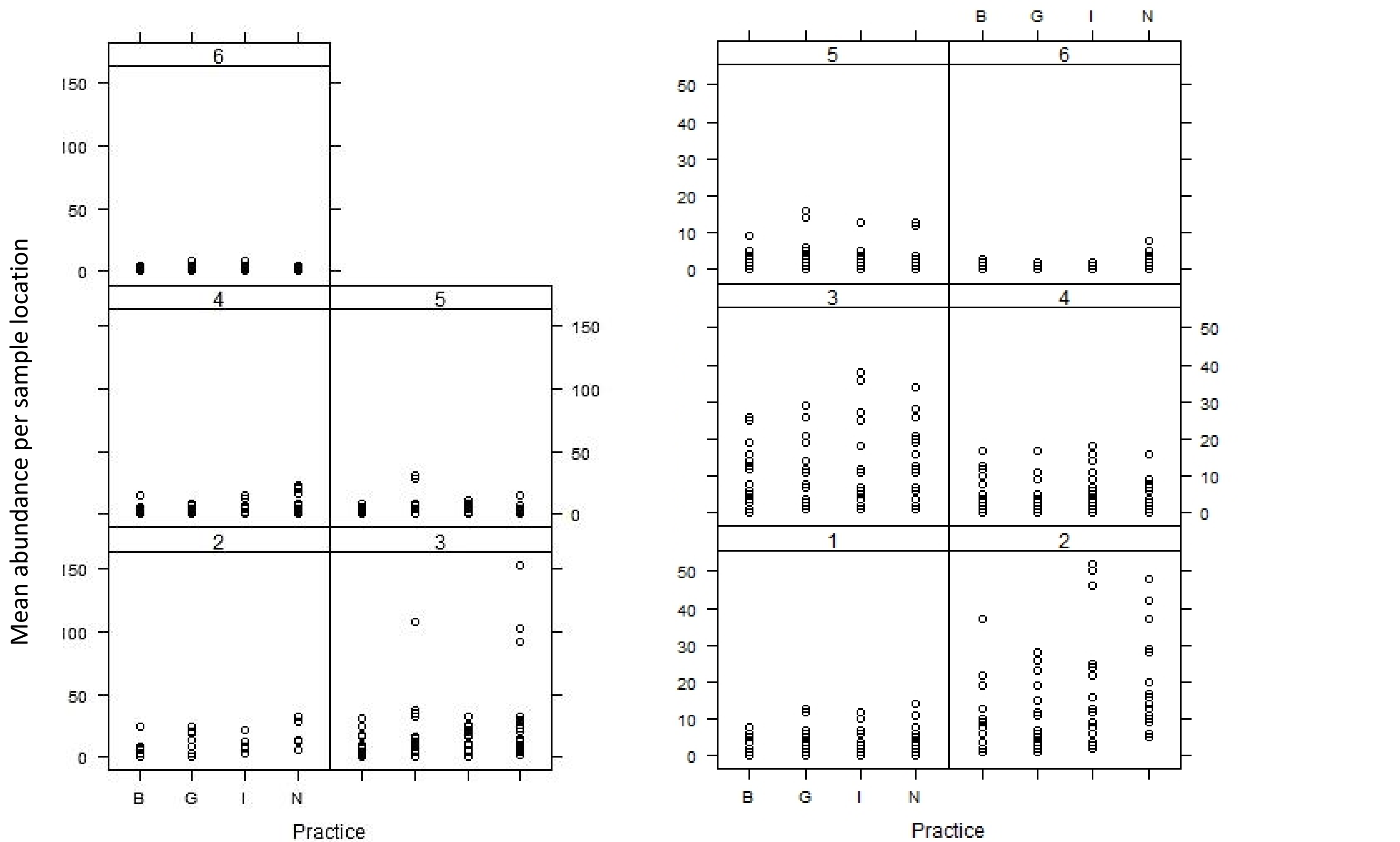



Figure 6. Mean abundance ground nesting bees captured at each location (a set of traps, represented as "plot" in the graph) during May (denoted as 1), June (2), July (3), August (4), September (5) and October (6) in 2011 and 2012. Practice: B, Grazed Bermudagrass pasture (Practice code # 528); G, Grazed native warm season pasture (Practice code # 528 & 550); I, Grazed Indiangrass, (Practice code # 528 & 550); N, Ungrazed native warm season pasture (Practice code # 550).

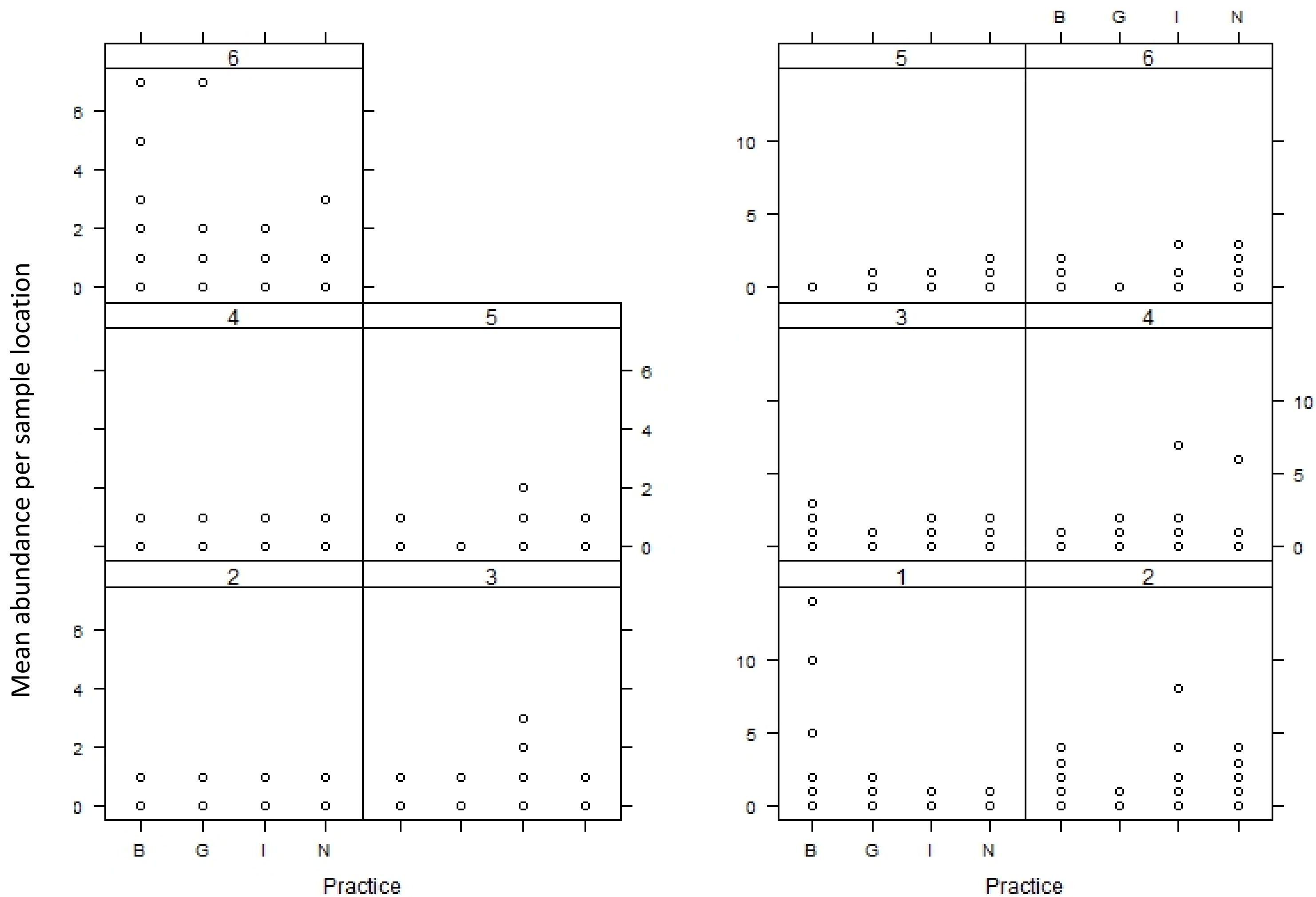



Figure 7. Mean abundance non-ground nesting (wood/stem/cavity nesting) bees captured at each location (a set of traps, represented as "sample location" in the graph) during May (denoted as 1), June(2), July(3), August(4), September(5) and October(6) in 2011 and 2012. Practice: B, Grazed Bermudagrass pasture (Practice code # 528); G, Grazed native warm season pasture (Practice code # 528 & 550); I, Grazed Indiangrass, (Practice code # 528 & 550); N, Ungrazed native warm season pasture (Practice code # 550).

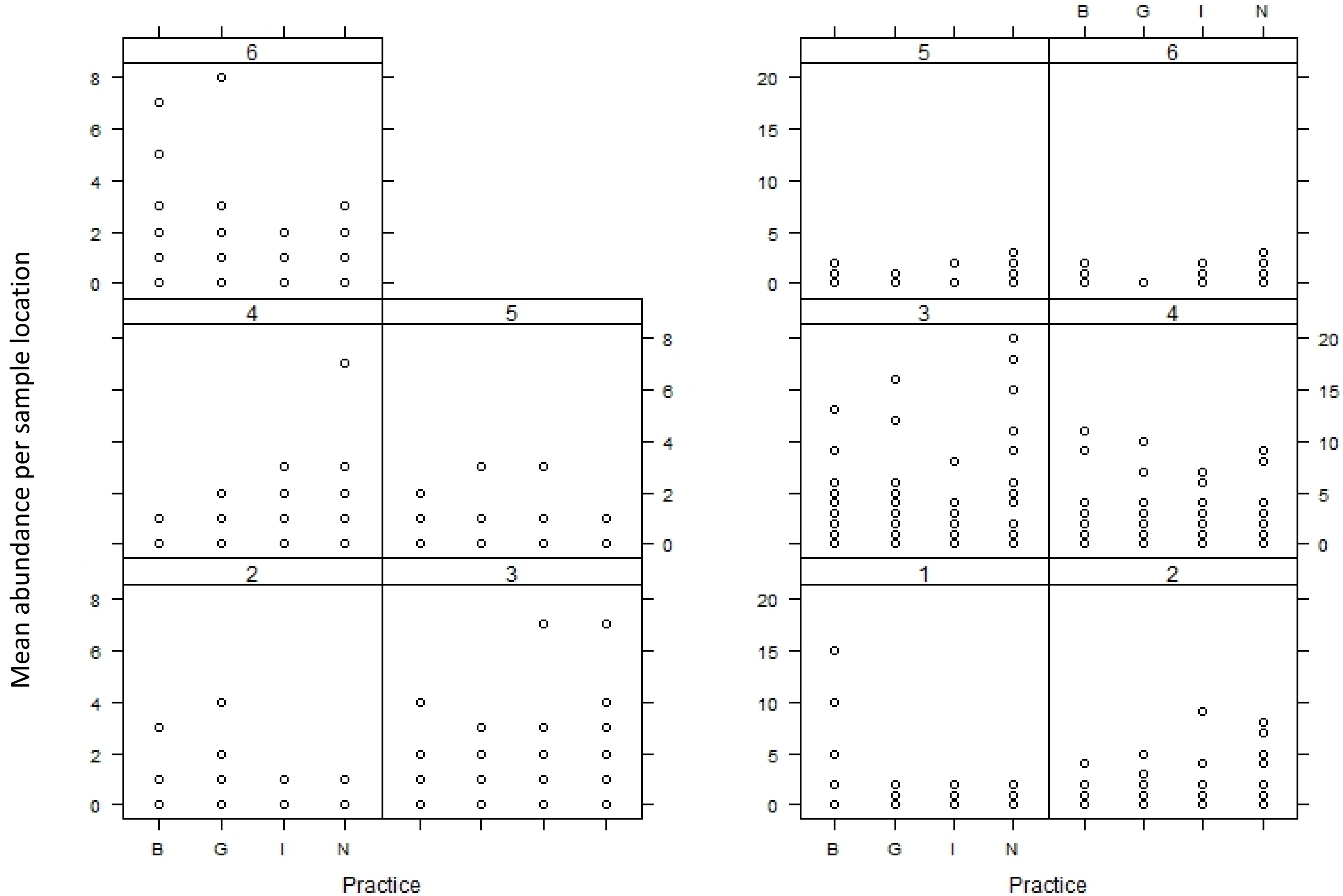



Figure 8. Mean abundance of bees of Apidae captured at each location (a set of traps, represented as "sample location" in the graph) during May (denoted as 1), June (2), July (3), August (4), September (5) and October (6) in 2011 and 2012. Practice: B, Grazed Bermudagrass pasture (Practice code # 528); G, Grazed native warm season pasture (Practice code # 528 & 550); I, Grazed Indiangrass, (Practice code # 528 & 550); N, Ungrazed native warm season pasture (Practice code # 550).

Table 5. Summary of F-values (P-values in parenthesis) from a general linear mixed model with practice as fixed effect and pasture as random effect. Significant practice effects (P<0.05) are bolded.

| Year | Response variable (Abundance) | Month | | | | | |
|---|---|---|---|---|---|---|---|
| | | May | June | July | August | September | October |
| 2011 | Total | – | 0.678 (0.593) | 1.343 (0.327) | 0.896 (0.484) | 0.079 (0.970) | 0.867 (0.497) |
| | Ground | – | 0.616 (0.626) | 1.364 (0.322) | 0.990 (0.445) | 0.0851 (0.966) | 0.280 (0.838) |
| | Non-ground | – | 1.421 (0.315) | 1.839 (0.218) | 0.190 (0.900) | 0.729 (0.563) | 2.903 (0.101) |
| | Apidae | – | 0.499 (0.695) | 0.709 (0.573) | 0.400 (0.757) | 0.057 (0.981) | 1.028 (0.430) |
| 2012 | Total | 0.494 (0.696) | **5.846 (0.021)** | 0.411 (0.750) | 0.449 (0.725) | 0.437 (0.733) | 1.605 (0.263) |
| | Ground | 0.886 (0.489) | **6.048 (0.019)** | 0.555 (0.659) | 0.465 (0.714) | 0.505 (0.690) | 2.043 (0.187) |
| | Non-ground | 0.871 (0.495) | 0.548 (0.663) | 0.377 (0.773) | 0.200 (0.894) | 0.739 (0.559) | 1.220 (0.364) |
| | Apidae | 0.753 (0.551) | 0.667 (0.596) | 1.432 (0.304) | 0.372 (0.776) | 2.056 (0.185) | 2.100 (0.179) |

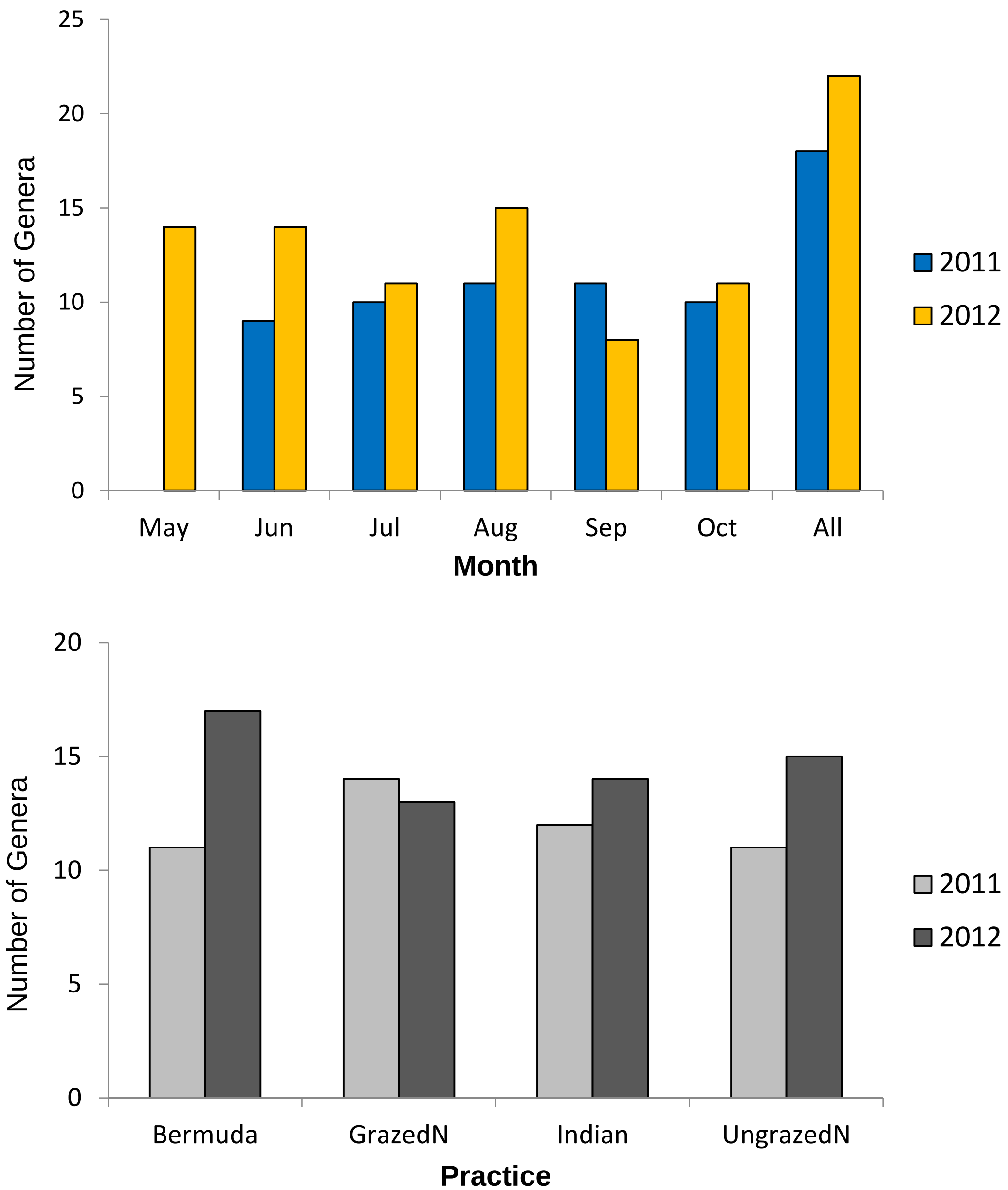



Figure 9. Genus richness (number of genera) of bees by month (upper) and practice (lower). “All” in the upper graph represents overall genus richness at each year, 2011 and 2012. Practice: Bermuda, Grazed Bermudagrass pasture (Practice code # 528); GrazedN, Grazed native warm season pasture (Practice code # 528 & 550); Indian, Grazed Indiangrass, (Practice code # 528 & 550); UngrazedN, Ungrazed native warm season pasture (Practice code # 550).

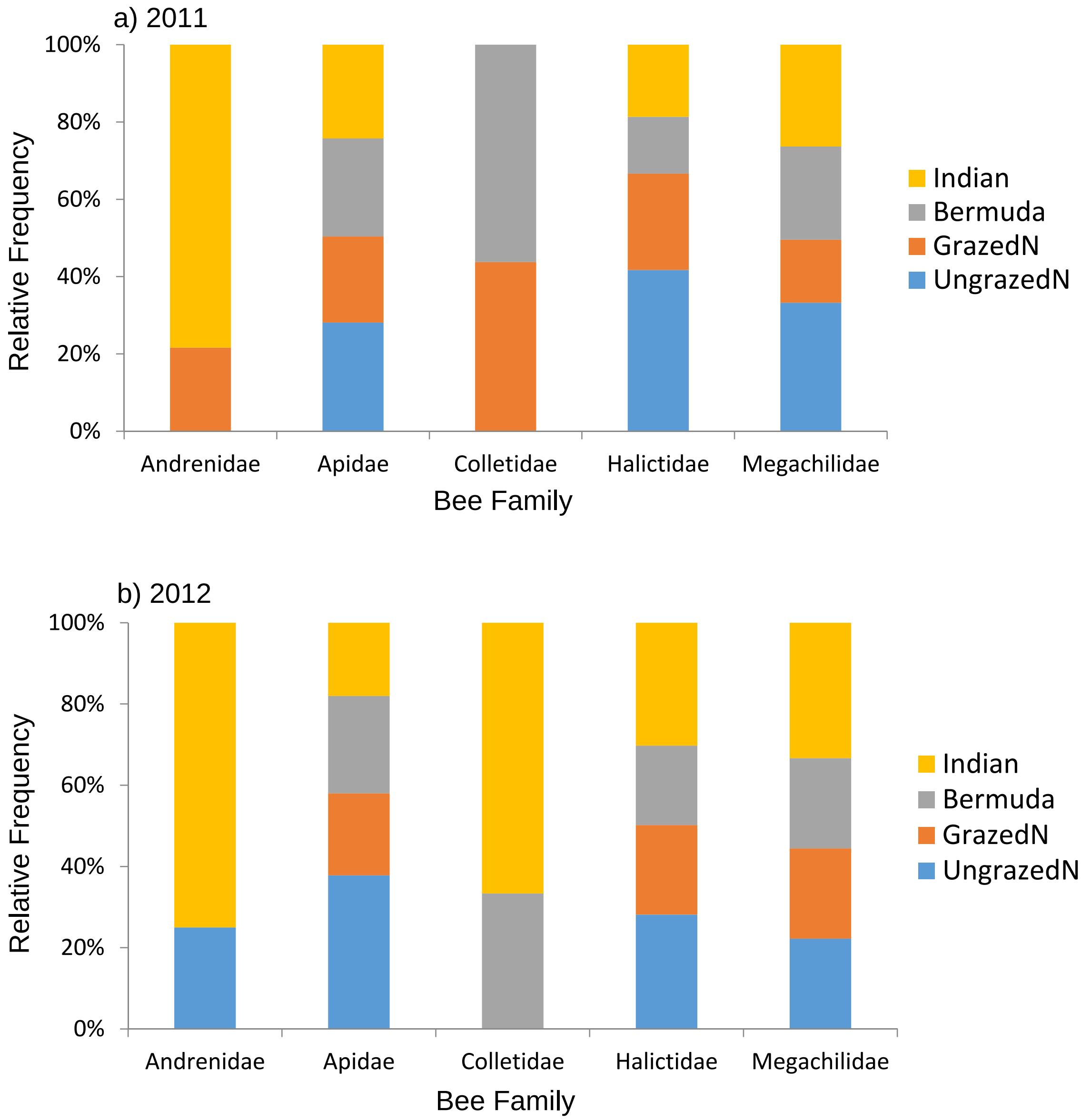
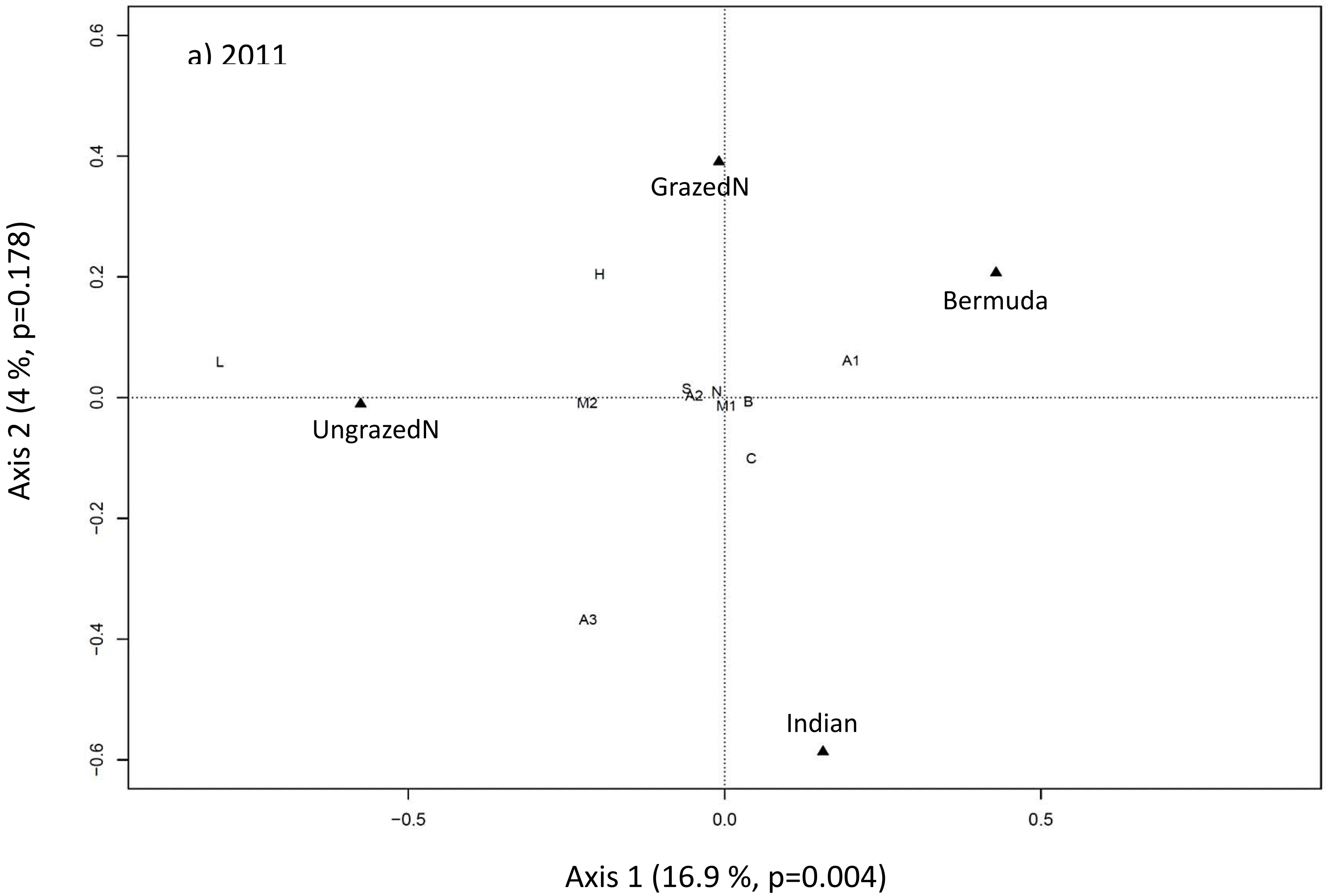



Figure 10. Relative proportion of mean abundance of five bee families at each practice. Note that abundance of Andrenidae and Colletidae was very low: $\leq 5$ total individuals were captured each year. Practice: Bermuda, Grazed Bermudagrass pasture (Practice code # 528); GrazedN, Grazed native warm season pasture (Practice code # 528 & 550); Indian, Grazed Indiangrass, (Practice code # 528 & 550); UngrazedN, Ungrazed native warm season pasture (Practice code # 550).



a) 2011
GrazedN
Bermuda
UngrazedN
Indian
H
L
A1
S
A2
N
M2
M1
B
C
A3
0.6
0.4
0.2
0.0
−0.2
−0.4
−0.6
−0.5
0.0
0.5
Axis 2 (4 %, p=0.178)
Axis 1 (16.9 %, p=0.004)

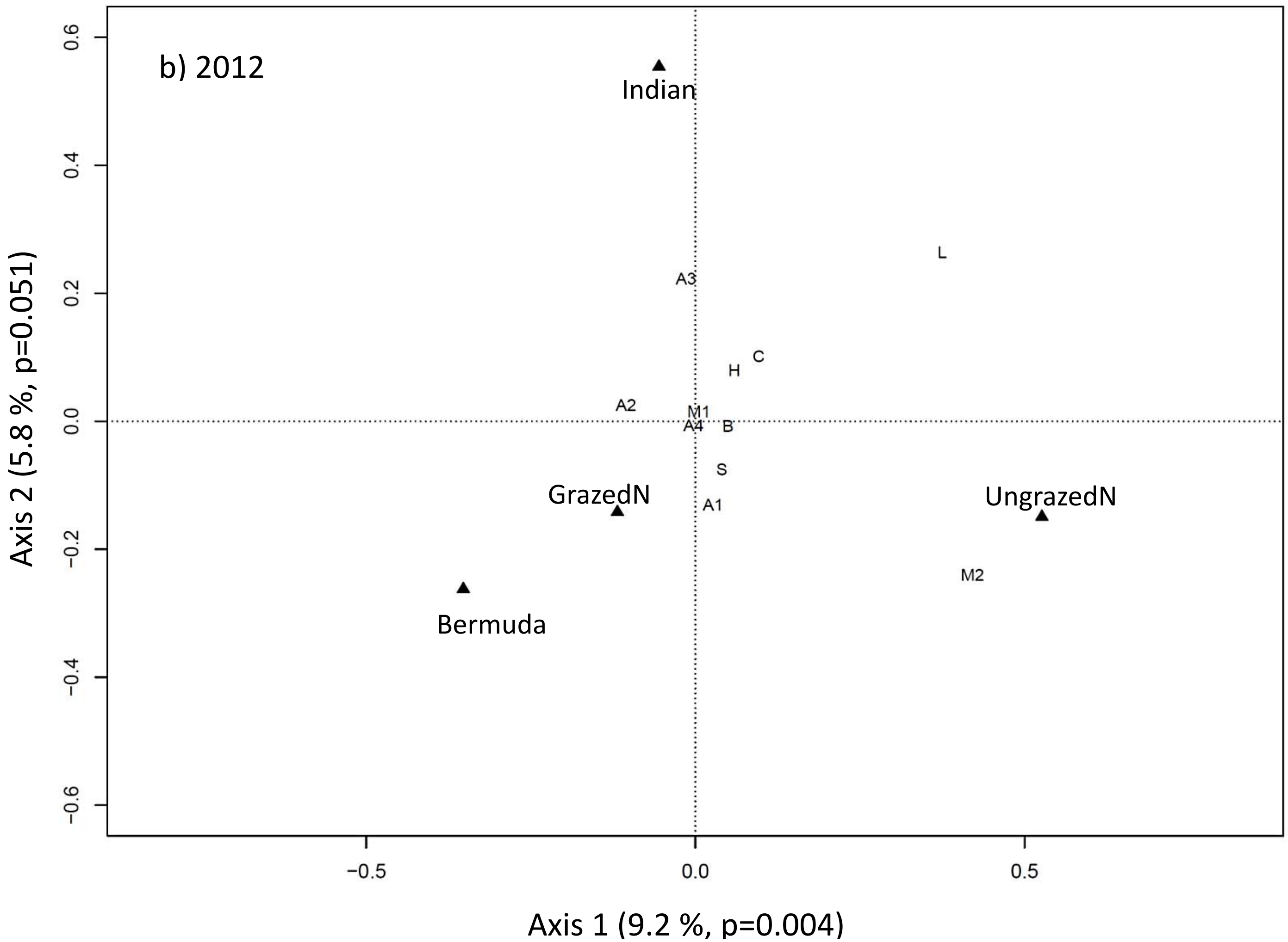



Figure 11. Redundancy analysis (RDA) biplot of four practices and bee genera in 2011(a) and 2012 (b). Variance (%) explained by each of the first two axis and *p* values calculated from permutation test of 999 iterations are shown in parenthesis. Practice: Bermuda, Grazed Bermudagrass pasture (Practice code # 528); GrazedN, Grazed native warm season pasture (Practice code # 528 & 550); Indian, Grazed Indiangrass, (Practice code # 528 & 550); UngrazedN, Ungrazed native warm season pasture (Practice code # 550). Genus: A1, Apis; A2, Augochlora; A3, Augochlorella; A4, Augochloropsis; B, Bombus; C, Certina; H, Halictus; L, Lasioglossum; M1, Megachile; M2, Melissodes; N, Nomia; S, Svastra.

Table 6. Order, family, and guild classification (based on foraging strategy) of all insects detected during 2011-2012, at Prairie site, Mississippi.

| **Order** | **Family** | **Foraging strategy** |
|---|---|---|
| Coleoptera | Buprestidae | Pollinators |
| | Cerambycidae | Herbivores |
| | Latridiidae | Others/fungivores |
| | Meloidae | Predators/parasites/parasitoids |
| | Mordellidae | Others/omnivores |
| | Scarabaeidae | Herbivores |
| Diptera | Bombyliidae | Pollinators, Predators/parasites/parasitoids |
| | Conopidae | Predators/parasites/parasitoids, Pollinators |
| | Dolichopodidae | Predators/parasites/parasitoids |
| | Stratiomyidae | Others/omnivores |
| | Syrphidae | Predators/parasites/parasitoids, Pollinators |
| Hymenoptera | Andrenidae | Pollinators |
| (Bees) | Apidae | Pollinators |
| | Colletidae | Pollinators |
| | Halictidae | Pollinators, Herbivores |
| | Megachilidae | Pollinators, Predators/parasites/parasitoids |
| Hymenoptera | Crabronidae | Predators/parasites/parasitoids |
| (Non-bees, | Sphecidae | Predators/parasites/parasitoids |
| Wasps) | Tiphiidae | Pollinators |
| | Vespidae | Predators/parasites/parasitoids |
| Lepidoptera | Hesperiidae | Pollinators, Herbivores |
| | Lycaenidae | Pollinators |
| | Nymphalidae | Pollinators |
| | Papilionidae | Pollinators |
| | Pieridae | Pollinators |

Table 7. Number of individuals of each family of insects captured at Prairie site, Mississippi during 2011-2012. Note that more trapping occasions were performed in 2012.

| **Family** | **2011** | | | | | | **2012** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Jun[1] | Jul | Aug | Sep[1] | Oct | **TOTAL** | May | Jun | Jul | Aug[2] | Sep | Oct | **TOTAL** |
| Conopidae | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dolichopodidae | 439 | 207 | 907 | 1092 | 1798 | 4443 | 10859 | 3857 | 568 | 8218 | 4434 | 4733 | 32669 |
| Bombyliidae | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Syrphidae | 252 | 74 | 4 | 31 | 368 | 729 | 384 | 157 | 2 | 4 | 18 | 58 | 623 |
| Stratiomyidae | 0 | 0 | 1 | 1 | 2 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Meloidae | 54 | 100 | 31 | 13 | 21 | 219 | 5 | 47 | 20 | 31 | 52 | 30 | 185 |
| Mordellidae | 41 | 35 | 9 | 2 | 5 | 92 | 0 | 2 | 4 | 6 | 0 | 0 | 12 |
| Latridiidae | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Buprestidae | 17 | 3 | 2 | 0 | 0 | 22 | 0 | 23 | 0 | 0 | 0 | 0 | 23 |
| Scarabaeidae | 0 | 1 | 2 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Cerambycidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 3 |
| Halictidae | 337 | 1283 | 274 | 189 | 103 | 2186 | 283 | 1003 | 590 | 289 | 175 | 53 | 2393 |
| Megachilidae | 5 | 1 | 0 | 3 | 3 | 12 | 2 | 4 | 2 | 0 | 0 | 1 | 9 |
| Andrenidae | 1 | 0 | 3 | 0 | 1 | 5 | 1 | 3 | 0 | 0 | 0 | 0 | 4 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Apidae | 17 | 66 | 37 | 23 | 64 | 207 | 48 | 105 | 267 | 166 | 27 | 20 | 633 |
| Colletidae | 0 | 0 | 1 | 0 | 1 | 2 | 2 | 0 | 0 | 1 | 0 | 0 | 3 |
| Hesperiidae | 4 | 30 | 21 | 22 | 19 | 96 | 5 | 32 | 16 | 20 | 33 | 36 | 142 |
| Lycaenidae | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 1 | 0 | 0 | 0 | 0 | 4 |
| Pieridae | 2 | 5 | 0 | 2 | 3 | 12 | 3 | 8 | 0 | 3 | 1 | 0 | 15 |
| Papilionidae | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 2 |
| Nymphalidae | 1 | 1 | 1 | 0 | 3 | 6 | 0 | 0 | 0 | 0 | 1 | 2 | 3 |
| Vespidae | 0 | 0 | 3 | 6 | 8 | 17 | 0 | 10 | 22 | 4 | 5 | 5 | 46 |
| Sphecidae | 0 | 4 | 1 | 3 | 7 | 15 | 1 | 2 | 8 | 2 | 0 | 1 | 14 |
| Crabronidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Tiphiidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| **TOTAL** | 1170 | 1810 | 1297 | 1390 | 2407 | 8074 | 11597 | 5258 | 1500 | 8746 | 4747 | 4939 | 36787 |

[1] only one trapping occasion
[2] three trapping occasions

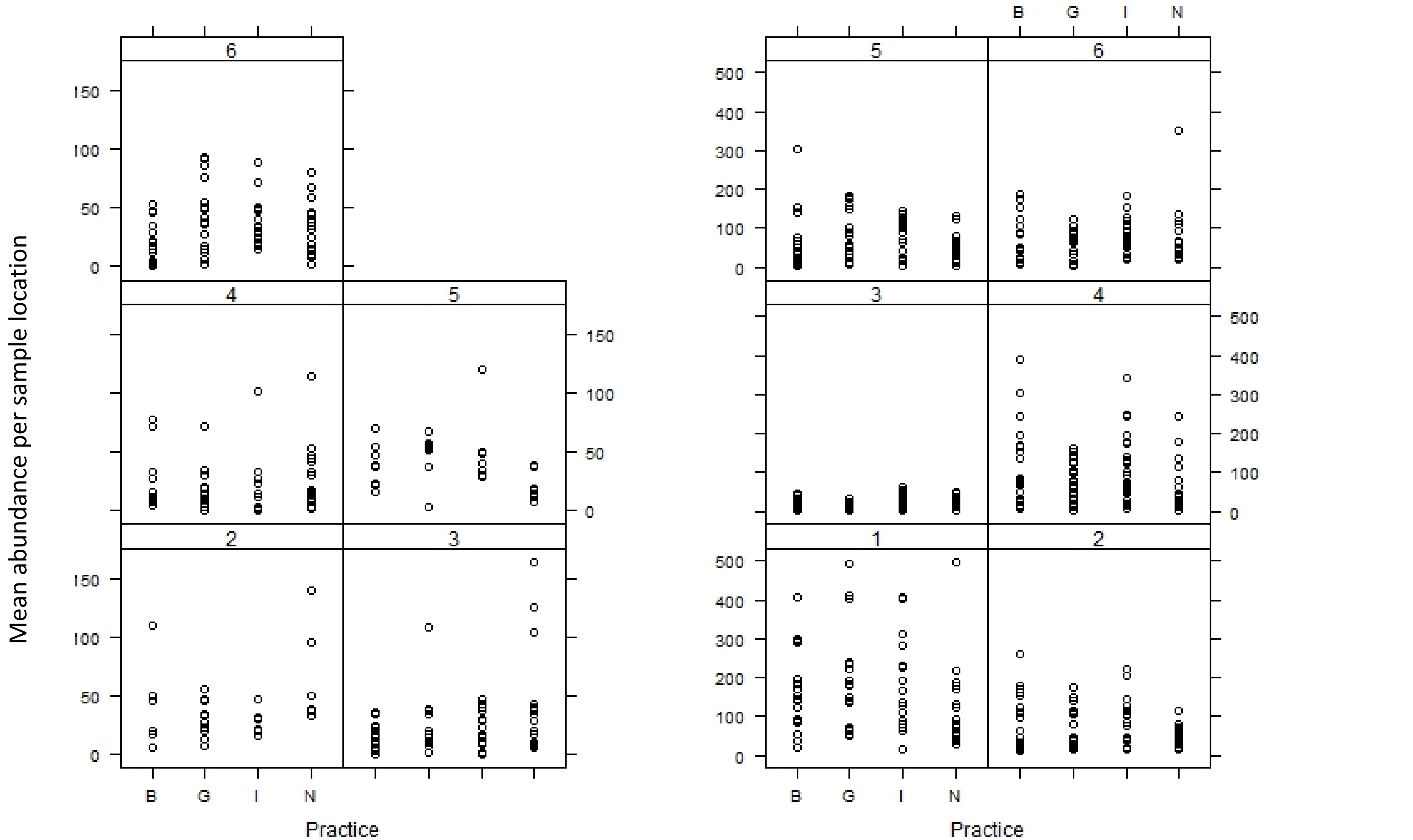



Figure 12. Mean abundance of all insects captured at each location (a set of traps, represented as “sample location” in the graph) during May (denoted as 1), June(2), July(3), August(4), September(5) and October(6) in 2011 (Left) and 2012 (Right). Practice: B, Grazed Bermudagrass pasture (Practice code # 528); G, Grazed native warm season pasture (Practice code # 528 & 550); I, Grazed Indiangrass, (Practice code # 528 & 550); N, Ungrazed native warm season pasture (Practice code # 550).

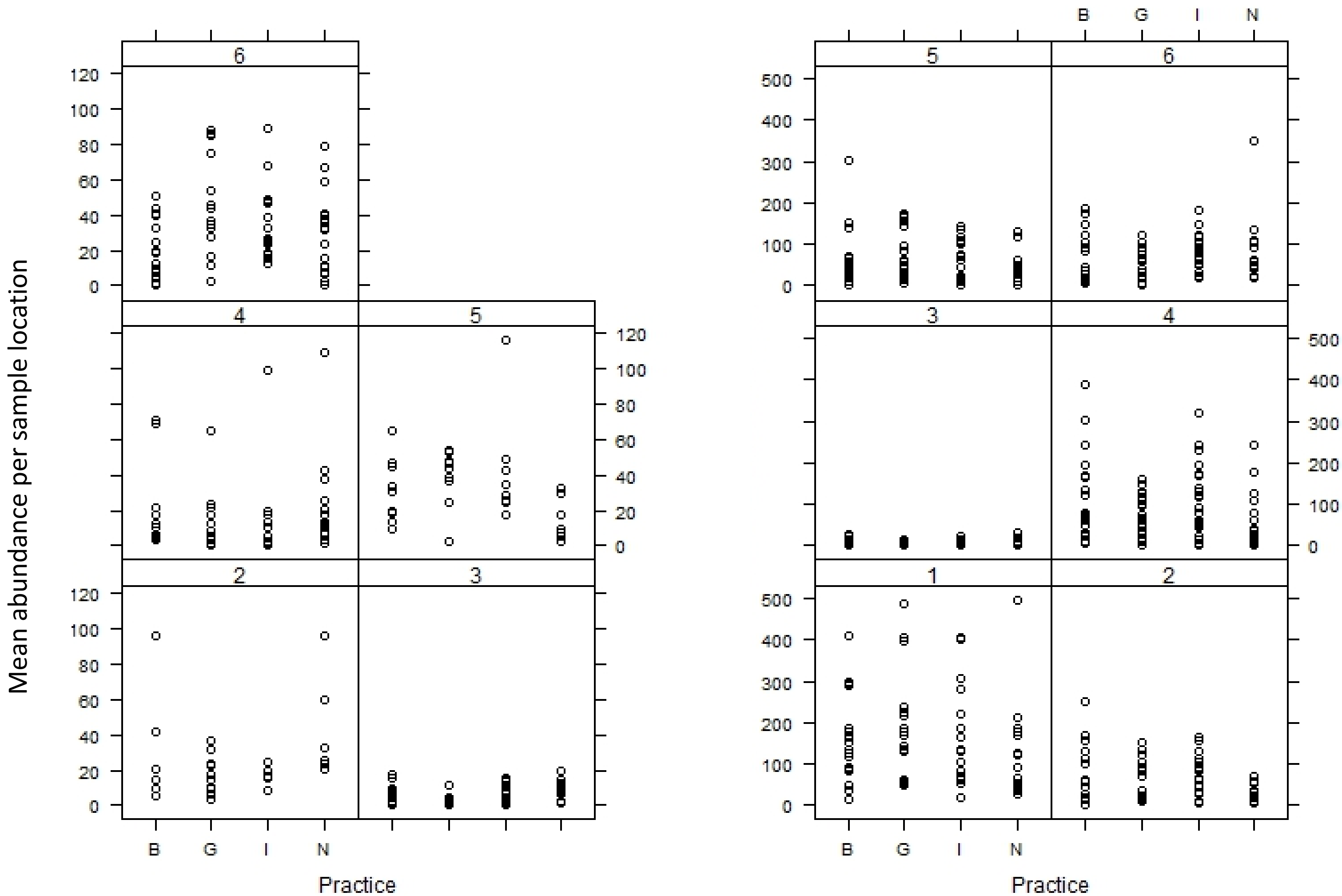



Figure 13. Mean abundance of predator/parasite insects captured at each location (a set of traps, represented as “sample location” in the graph) during May (denoted as 1), June(2), July(3), August(4), September(5) and October(6) in 2011 (Left) and 2012 (Right). Practice: B, Grazed Bermudagrass pasture (Practice code # 528); G, Grazed native warm season pasture (Practice code # 528 & 550); I, Grazed Indiangrass, (Practice code # 528 & 550); N, Ungrazed native warm season pasture (Practice code # 550).

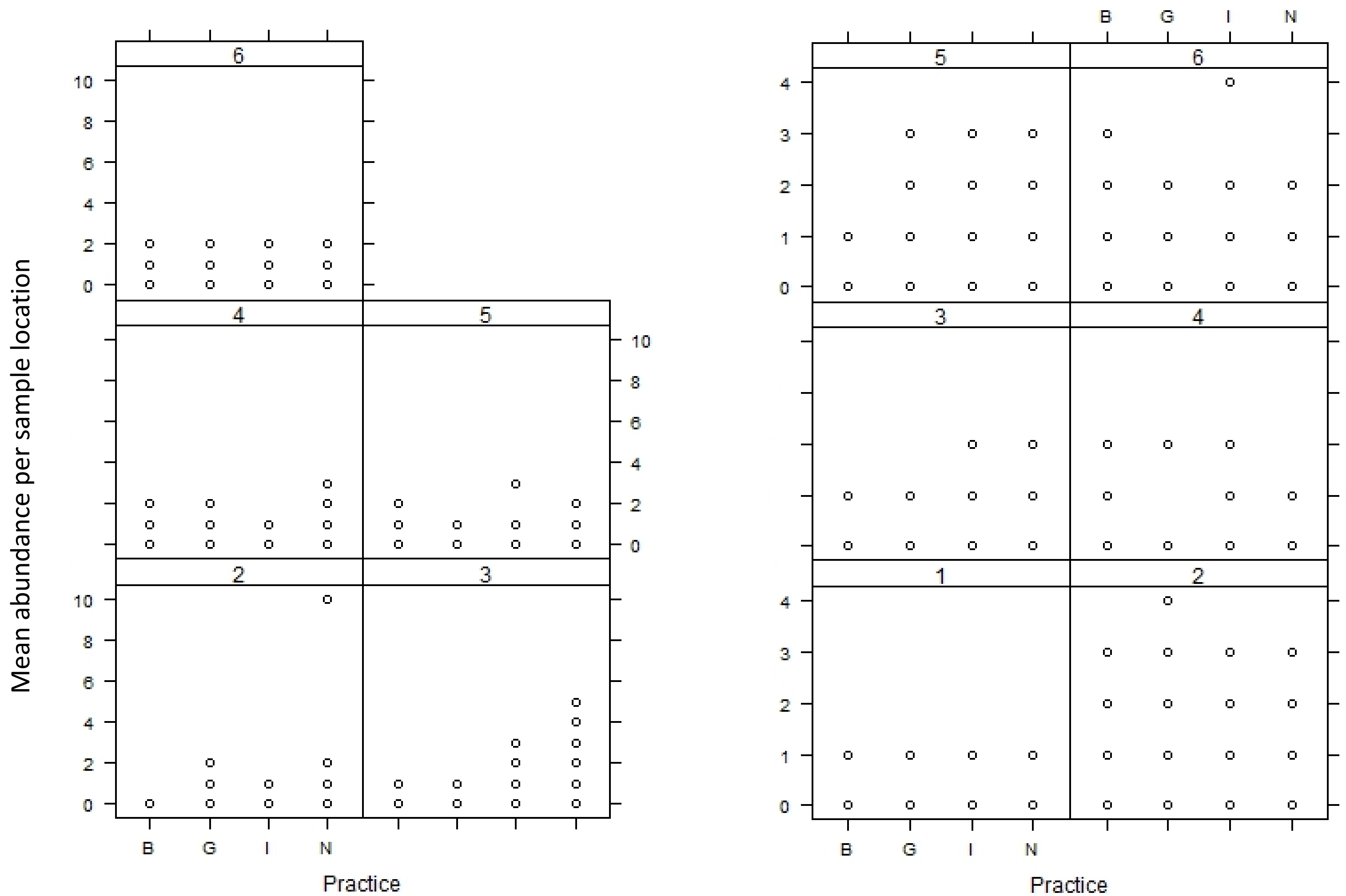



Figure 14. Mean abundance of herbivore insects captured at each location (a set of traps, represented as "sample location" in the graph) during May (denoted as 1), June(2), July(3), August(4), September(5) and October(6) in 2011 (Left) and 2012 (Right). Practice: B, Grazed Bermudagrass pasture (Practice code # 528); G, Grazed native warm season pasture (Practice code # 528 & 550); I, Grazed Indiangrass, (Practice code # 528 & 550); N, Ungrazed native warm season pasture (Practice code # 550).

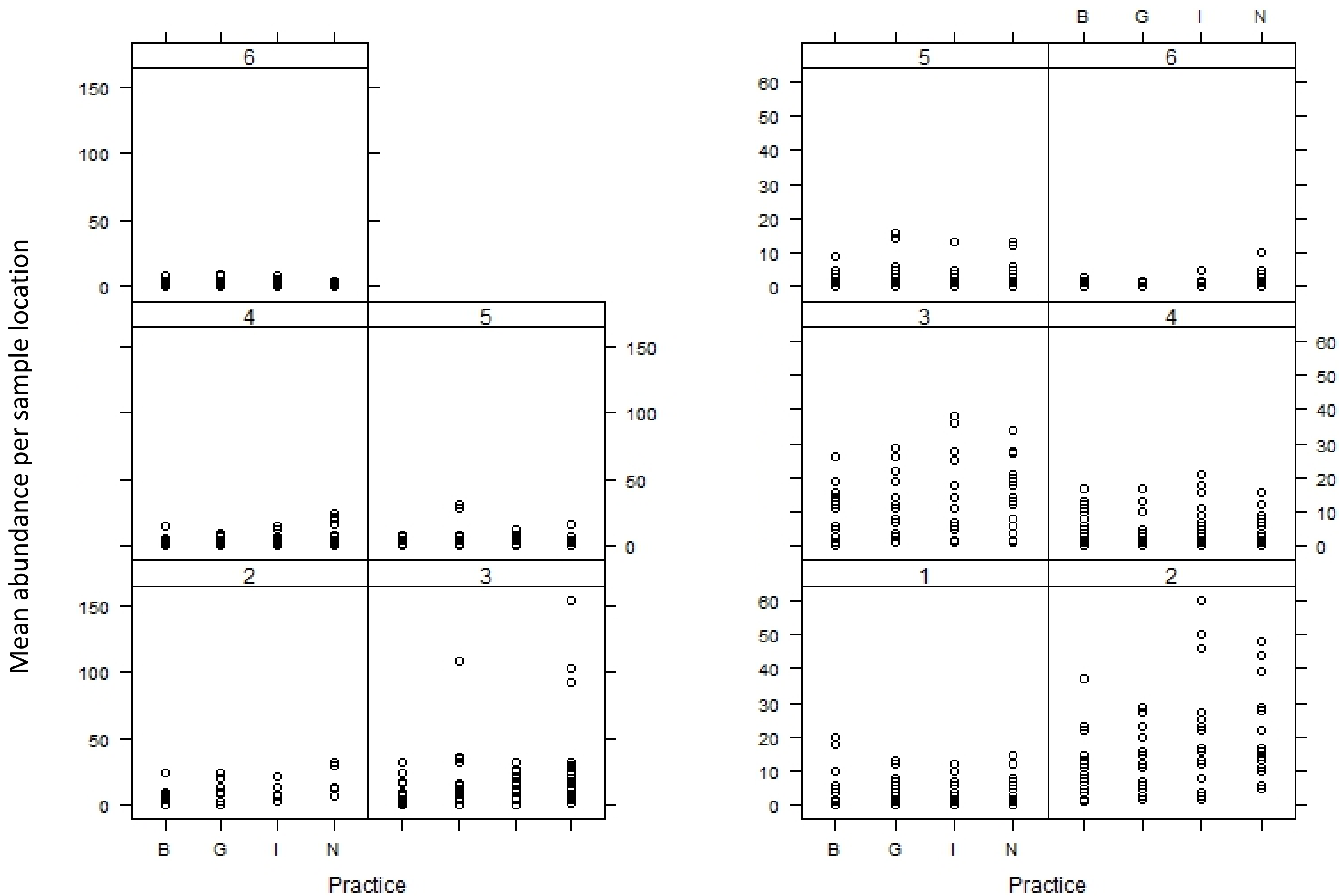



Figure 15. Mean abundance of pollinator insects captured at each location (a set of traps, represented as "sample location" in the graph) during May (denoted as 1), June(2), July(3), August(4), September(5) and October(6) in 2011 (Left) and 2012 (Right). Practice: B, Grazed Bermudagrass pasture (Practice code # 528); G, Grazed native warm season pasture (Practice code # 528 & 550); I, Grazed Indiangrass, (Practice code # 528 & 550); N, Ungrazed native warm season pasture (Practice code # 550).

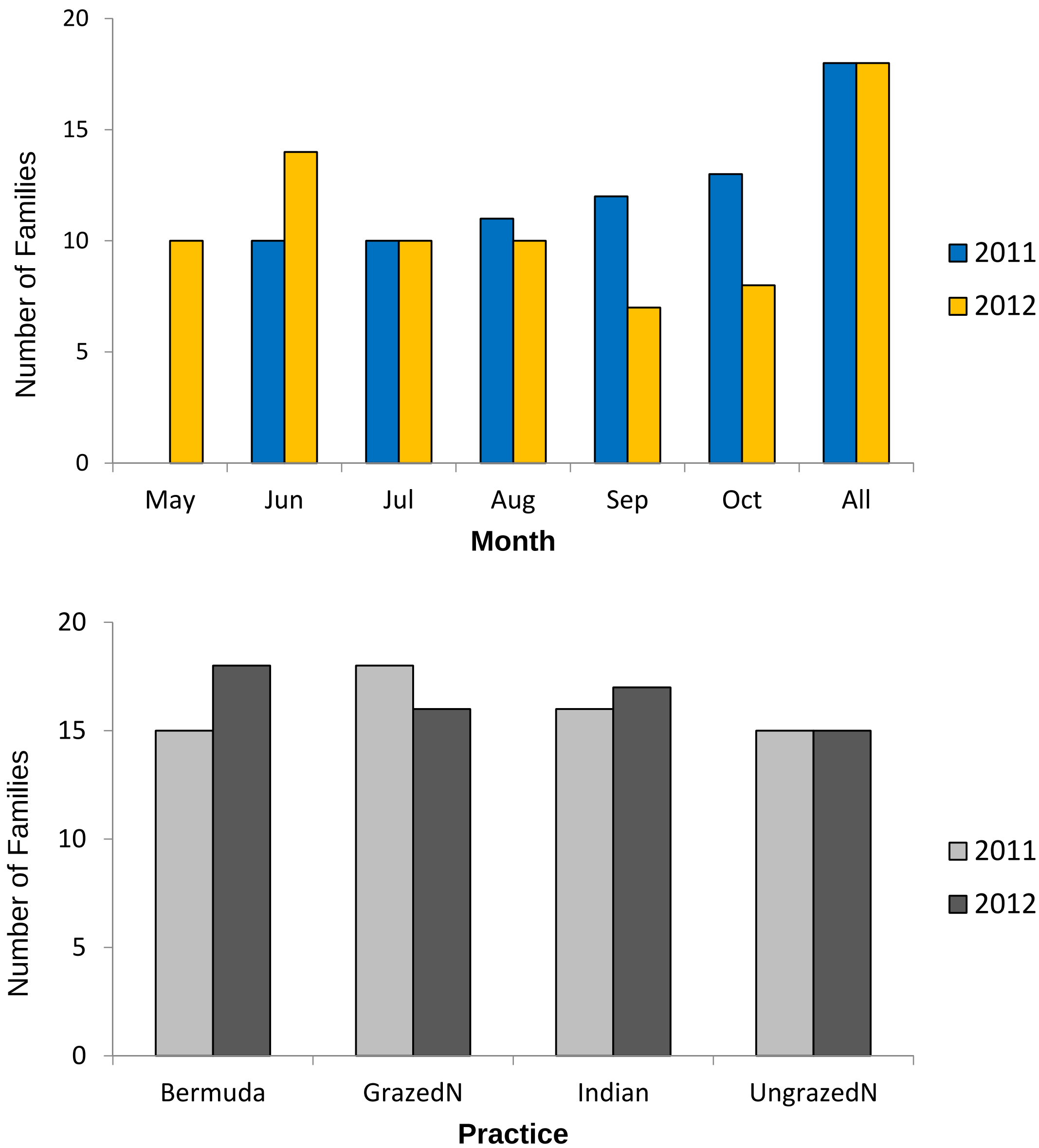



Figure 16. Family richness (number of families) of insects by month (upper) and practice (lower). "All" in the upper graph represents overall family-level richness at each year, 2011 and 2012. Practice: Bermuda, Grazed Bermudagrass pasture (Practice code # 528); GrazedN, Grazed native warm season pasture (Practice code # 528 & 550); Indian, Grazed Indiangrass, (Practice code # 528 & 550); UngrazedN, Ungrazed native warm season pasture (Practice code # 550).

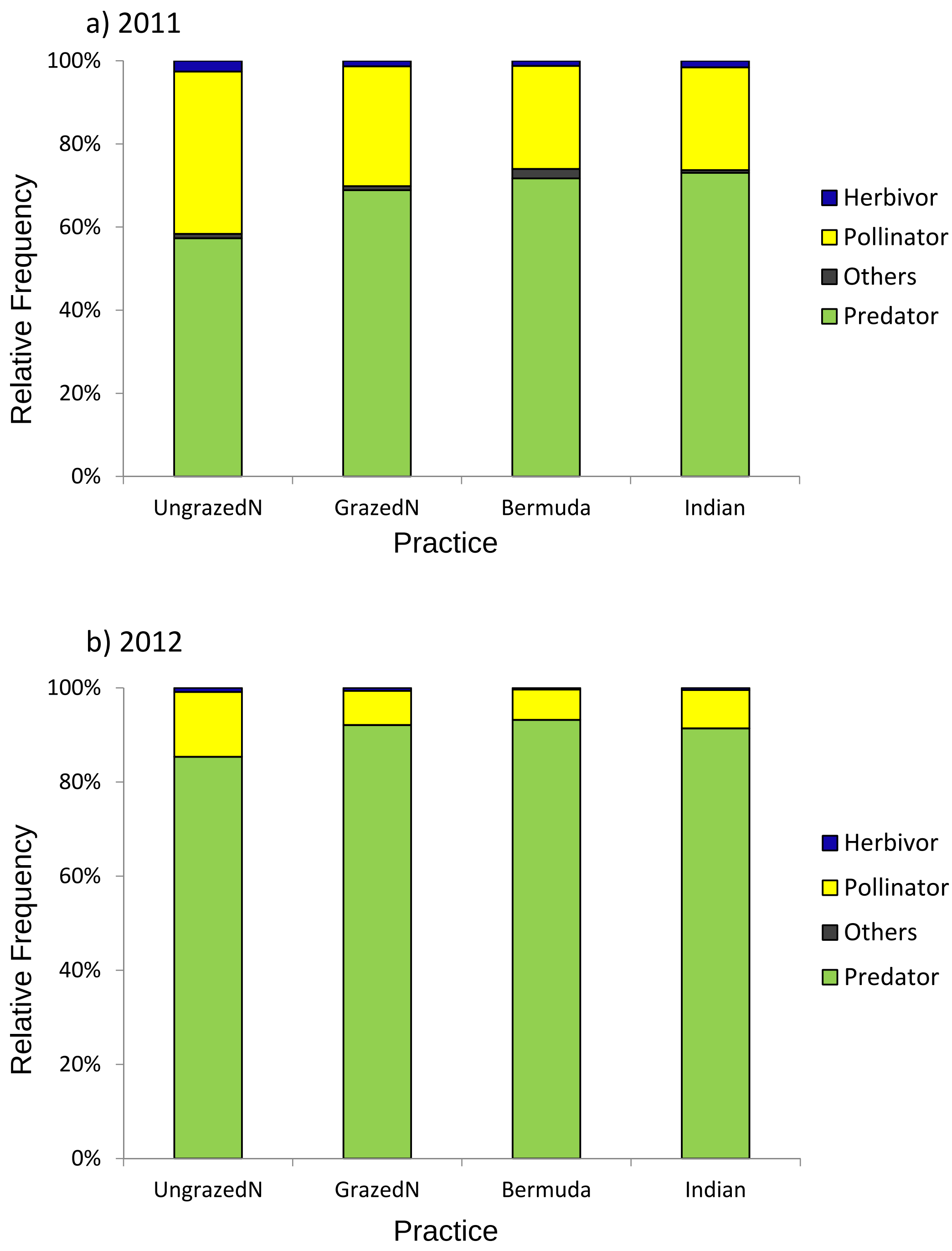



Figure 17. Relative proportion of mean abundance of insect foraging guilds at each practice. "Others" includes fungivores and omnivores. "Predator" represents predators/parasites/parasitoids foraging guild. Practice: Bermuda, Grazed Bermudagrass pasture (Practice code # 528); GrazedN, Grazed native warm season pasture (Practice code # 528 & 550); Indian, Grazed Indiangrass, (Practice code # 528 & 550); UngrazedN, Ungrazed native warm season pasture (Practice code # 550).

## 2. Bryan Farms Site

### 2.1. Bees

#### 2.1.1. General pattern

A total of 21571 bees of 22 genera were captured during 2011-2012 (Table 8 and Table 9). Lasioglossum was the most abundant, comprising 91% of total bees in 2011 and 86% in 2012. Abundance of bees of Apidae accounted for 5.6% of total bees in 2011 and increased 1.7 times in 2012, occupying 11.6% of total bees. There were variations in bee abundance between months. Abundance of most bee genera peaked during late spring-mid summer, particularly in June and then decreased throughout late-summer and fall (Table 9 and Fig.18-21). However, Apis, Halictus and Lasiolossum were also abundant during November in 2011 and Lasiolossum during October in 2012. Svastra were the most abundant in late summer (2012) or early fall (2011).

Total bees and ground-nesting bees showed high mean abundance in June but low in October both years (Fig. 18-19). Mean abundance of non-ground nesting bees tended to be low in August (2011) and September (2012) (Fig. 20). Mean abundance of Apidae was high in June and September in 2011 and August in 2012 (Fig. 21).

#### 2.1.2. Effect of practice

*Abundance*

Like the pattern observed at Prairie site, mean abundance of bees varied more between locations or months than practices (Fig. 18-21). While mean abundance of bees (total, ground nesting, non-ground nesting, and Apidae) was similar among practice in 2012, there were some variations between NWGS and Switchgrass in 2011. Mean abundance of total bees and ground-nesting bees tended to be higher at NWGS (NM and NS) than Switchgrass (SM and SS) during July-September and November, whereas the opposite pattern was observed during May-June.

Significant practice effect was found in 2011: abundance of total bees and ground-nesting bees in June and November and week effect on abundance of Apidae in August (Table 10). On the other hand, no significant effect was observed in 2012.

*Genus richness*

Monthly genus richness and overall genus richness was greater in 2011 than in 2012 except May, July and August (Fig. 22). In particular, genus richness of 2011 was two times greater in September. However, these patterns may be influenced by unequal number of sample locations between years. Effect of practice on genus richness did not vary with year. Genus richness was the same at NWSG S and Switch S, and slightly greater at NWSG M (2011) and Switch M (2012) than other two practices.

*Composition of bee community*

At family level, Apidae and Halictidae were evenly abundant across all practices both years (Fig. 23). Although only 2 Andrenidae bees were captured in 2011, all of them were found at Switch M. While Colletidae was more abundant at NWGS S both years, Megachilidae tended to be abundant at NWGS M in 2012.

At genus level, dissimilarity in bee genus composition among four practices was observed in 2011 (ANOSIM statistic $R = 0.12$, $p = 0.003$) whereas no significant difference in bee community composition was found in 2012 (ANOSIM statistic $R = 0.04$, $p = 0.14$). In addition, low value of R in 2011 indicates that the dissimilarity in bee community among practices is weak.

Similarly, the results of RDA showed low associations between practices and genera: practice explained < 15% (2011) and 11% (2012) of variations in the data. However, global permutation test of the RDA result of 2012 was not significant ($p= 0.112$), suggesting that overall association is insignificant in 2012. Axis 1 represented a gradient from NWSG (especially, NWSG S) to Swtichgrasses in 2011, whereas it represented a gradient from single harvest to multiple harvests in 2012. Although this pattern is consistent with timing of harvest practice (no harvest in 2011 and harvest in 2012), separation of NWSG M from NWSG S (Axis 1) in 2011 may indicate some variations between NWSG plantings, which are not related to practices applied in this study. Most genera did not show clear associations with practices. Halictus (H1) was positively related to NWSG S in 2011. In 2012, Lasioglossum (L) and H1 showed positive association with a single harvest (Switch S and NWSG S) and Augochlorella with multiple harvests (Switch M and NWSG M) (Fig. 24).

## 2.2. Insects

### 2.2.1. General pattern

Over 79237 insects from 28 families were trapped during 2011-2012 (Table 11 and Table 12). Total abundance of all insects was similar between two years. Among 28 families, Dolichopodidae and Halictidae were the most dominant both years, comprising 87% of total insects in 2011 and 93% in 2012. Total insect abundance peaked in September and October in 2011 and May and June in 2012. This pattern was largely driven by above two families.

### 2.2.2. Effect of practice

*Abundance*

Mean abundance of total insects and of each foraging guild varied by location and month (Fig. 25-28). However, mean abundance among practices was also different. In 2011, mean abundance of total insects, predator/parasite/parasitoid insects, and pollinator insects was low at NWSG practices (NM and NS) during May-June, but high in November. Herbivore insects showed low mean abundance at Switchgrass practices (SM and SS). These patterns slightly changed in 2012. While mean abundance of total insects and predator/parasite/parasitoid insects was low at NM during May-June and August, it was high at NS. Both practices also showed high mean abundance of total insects and predators/parasites/parasitoids in October. Compared to 2011, mean abundance of herbivores was low in all practices. Although mean abundance of pollinators did not vary with practices, it was relatively low at NM in May.

*Family richness*

Overall family richness was high in 2011, however, family richness of insects varied by month (Fig. 29). During May-August, family richness was greater in 2012 than in 2011. While family richness decreased from summer to late fall in 2012, family richness was low during July-August in 2011. In 2012, family richness was greater in June and October. Among four practices, greater family richness was observed at NWSG (both single and multiple harvests) in 2011 and NWSG S in 2012. Switch S showed lower family richness both years.

*Composition of insect community*

Predator/parasite/parasitoid foraging guild was the most dominant in all practices (Fig. 30). Pollinators were the second dominant, particularly relatively higher proportion of pollinators was observed at NWGS M both years. Compared to other practices, insect community at NWSG practices included more other guilds than predator/parasite/parasitoid foraging guild both years, indicating relatively higher diversity in community structure.

Table 8. Family, genus (and species), and guild classification (based on nest placement) of bees detected during 2011-2012, at Bryan Farms site, Mississippi.

| **Family** | **Genus** | **Species** | **Nest placement guild** |
|---|---|---|---|
| Andrenidae | Andrena | | Ground nesting |
| Apidae | Anthophora | | Ground nesting |
| | Apis | | Eusocial hive[a] |
| | | *Apis mellifera* | |
| | Bombus | | Ground nesting |
| | | *Bombus griseocollis* | |
| | | *Bombus pennsylvanicus* | |
| | | *Bombus bimaculatus* | |
| | | *Bombus impatiens* | |
| | | *Bombus affinis* | |
| | | *Bombus auricomus* | |
| | | *Bombus citrinus* | |
| | | *Bombus fraternus* | |
| | | *Bombus fervidus* | |
| | Ceratina | | Wood/Stem nesting |
| | | *Ceratina dupla* | |
| | | *Ceratina strenua* | |
| | | 2 Unknown Certina spp. | |
| | Eucera | | Ground nesting |
| | | *Eucera hamata* | |
| | Melissodes | | Ground nesting |
| | | *Melissodes bimaculata* | |
| | | *Melissodes tepaneca* | |

| | | | |
|---|---|---|---|
| | | *Melissodes comptoides* | |
| | | *Melissodes denticulata* | |
| | | *Melissodes trinodis* | |
| | | 4Unknown Melissodes spp.[b] | |
| | Melitoma | | Ground nesting |
| | | *Melitoma taurea* | |
| | Svastra | | Ground nesting |
| | | *Svastra atripes* | |
| | | *Svastra obliqua* | |
| | Triepeolus | | Ground nesting (Cleptoparasite) |
| | | *Triepeolus quadrifasciatus atlanticus* | |
| | | *Triepeolus simplex* | |
| | | *Tripeolus distinctus* | |
| | Xylocopa | | Wood nesting |
| | | *Xylocopa virginica* | |
| | | *Xylocopa mican* | |
| Colletidae | Hylaeus | | Stem nesting |
| | | *Hylaeus mesillae* | |
| | | *Hylaeus affinis* | |
| | | 1Unknown Hylaeus spp. | |
| Halictidae | Agapostemon | | Ground nesting |
| | | *Agapostemon virescens* | |
| | | *Agapostemon splendens* | |
| | Augochlora | | Wood nesting |
| | Augochlorella | | Ground nesting |
| | Augochloropsis | | Ground nesting |

| | | | |
|---|---|---|---|
| | | *Augochloropsis metallica* | |
| | Nomia | | Ground nesting |
| | Halictus | | Ground nesting |
| | | *Halictus poeyi* | |
| | | *Halictus parallelus* | |
| | | *Halictus rubicundus* | |
| | Lasioglossum[c] | | Ground nesting |
| Megachilidae | Coelioxys | | Wood/Stem nesting |
| | | *Coelioxys mexicana* | |
| | Lithurgus | | Wood/Stem nesting |
| | Megachile | | Wood/Stem nesting |
| | | *Megachile sculpturalis* | |
| | | *Megachile mimica* | |
| | | *Megachile mendeca* | |
| | | *Megachile integra* | |
| | | *Megachile brevis* | |
| | | *Megachile frugalis* | |
| | | *Megachile rotundata* | |
| | | 1Unknown Megachile spp. | |
| | Osmia | | Wood/Stem nesting |
| | | *Osmia conjuncta* | |
| | | *Osmia atriventris* | |
| | | *Unknown Osmia* spp. | |

[a] Considered as non- ground nesting for analysis.
[b] One of the species could be *M. communis* or *M. triodis*.
[c] Included a variety of species (e.g., *L. bruneri, L. callidum, L. mitchelli, L. pruninosum*, etc.).

Table 9. Number of individuals of each of bee genera detected at Bryan Farms site, Mississippi during 2011-2012.

| **Genus** | **2011** | | | | | | | | **2012** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | May[1] | Jun | Jul | Aug[1] | Sep[1] | Oct | Nov[1] | **TOTAL** | May | Jun[1] | Jul | Aug[2] | Sep[1] | Oct[1] | **TOTAL** |
| Agapostemon | 0 | 4 | 10 | 1 | 0 | 0 | 5 | 20 | 6 | 1 | 18 | 7 | 0 | 6 | 32 |
| Andrena | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Anthophorula | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Apis mellifera | 2 | 5 | 4 | 4 | 3 | 7 | 27 | 52 | 25 | 38 | 14 | 20 | 2 | 25 | 147 |
| Augochlora | 32 | 178 | 37 | 3 | 7 | 1 | 0 | 258 | 0 | 0 | 4 | 7 | 0 | 0 | 11 |
| Augochlorella | 5 | 22 | 4 | 8 | 9 | 8 | 67 | 123 | 24 | 106 | 3 | 2 | 0 | 24 | 136 |
| Augochloropsis | 4 | 1 | 0 | 0 | 0 | 0 | 7 | 12 | 4 | 3 | 2 | 1 | 0 | 4 | 10 |
| Bombus | 4 | 6 | 2 | 3 | 0 | 2 | 2 | 19 | 9 | 15 | 9 | 23 | 0 | 9 | 68 |
| Ceratina | 0 | 15 | 5 | 2 | 1 | 0 | 0 | 23 | 0 | 9 | 6 | 0 | 0 | 0 | 15 |
| Coelioxys | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Eucera | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Halictus | 29 | 49 | 21 | 4 | 22 | 12 | 208 | 345 | 26 | 42 | 20 | 15 | 0 | 26 | 106 |
| Hylaeus | 12 | 7 | 0 | 0 | 0 | 5 | 1 | 25 | 0 | 0 | 1 | 1 | 0 | 0 | 4 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lasioglossum | 1916 | 3556 | 2332 | 403 | 868 | 205 | 945 | 10225 | 1820 | 5084 | 920 | 463 | 72 | 1820 | 8412 |
| Lithurgus | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 |
| Megachile | 0 | 1 | 2 | 0 | 1 | 4 | 4 | 12 | 1 | 2 | 1 | 1 | 1 | 1 | 13 |
| Melissodes | 0 | 156 | 63 | 17 | 20 | 21 | 13 | 290 | 26 | 63 | 343 | 67 | 6 | 26 | 513 |
| Melitoma | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 1 | 0 | 0 | 4 |
| Nomia | 0 | 0 | 0 | 0 | 3 | 0 | 10 | 13 | 0 | 4 | 3 | 4 | 0 | 0 | 11 |
| Osmia | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 |
| Svastra | 1 | 4 | 23 | 42 | 163 | 0 | 0 | 233 | 0 | 4 | 148 | 221 | 8 | 0 | 381 |
| Tripeolus | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 2 | 0 | 0 | 4 |
| Xylocopa | 7 | 12 | 6 | 2 | 1 | 6 | 0 | 34 | 1 | 5 | 4 | 0 | 0 | 1 | 10 |
| **TOTAL** | 2013 | 4017 | 2510 | 489 | 1100 | 271 | 1291 | 11691 | 1944 | 5378 | 1500 | 835 | 89 | 1944 | 9880 |

[1]Only one trapping occasion
[2]Three trapping occasions

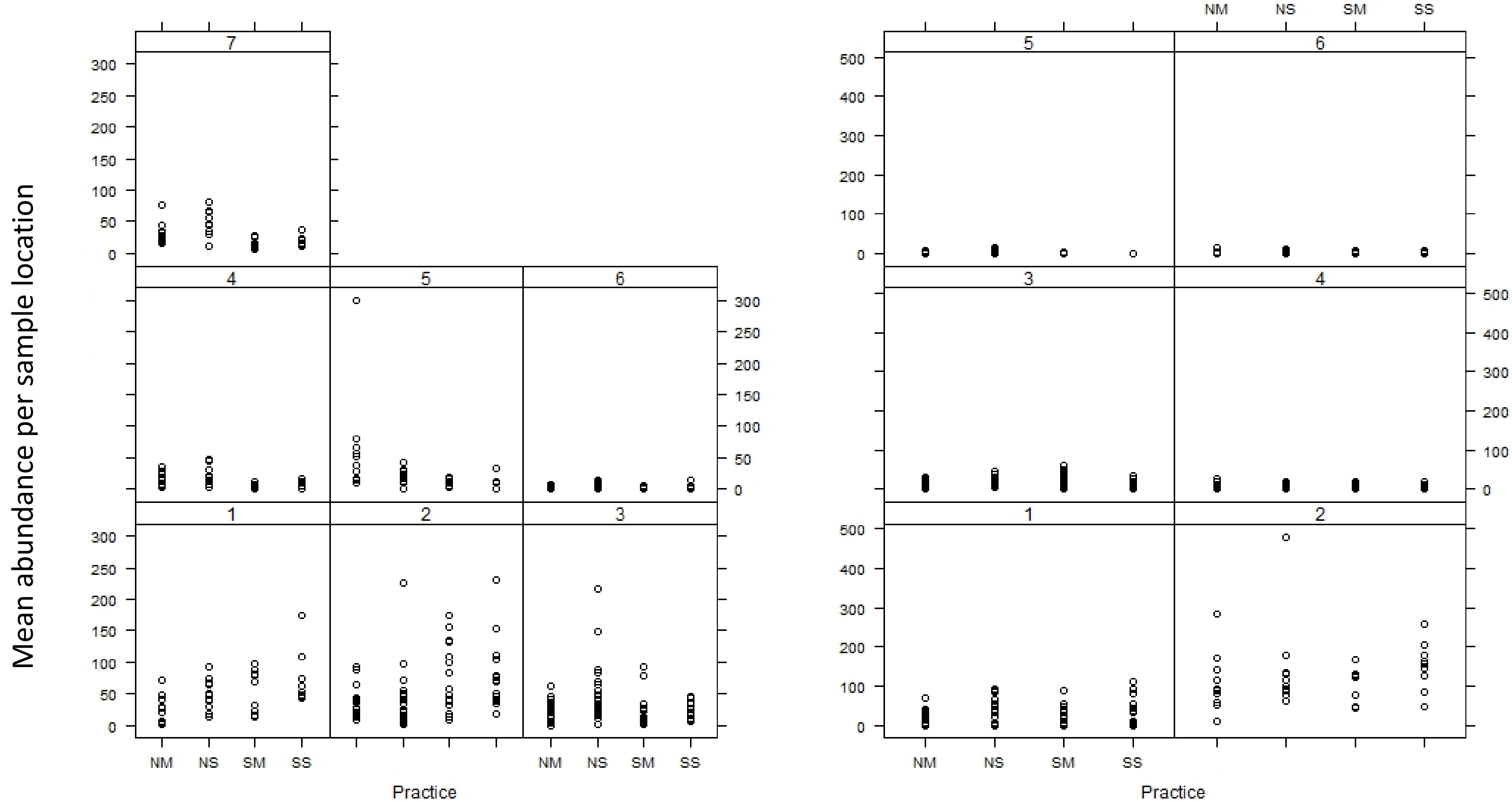



Figure 18. Mean abundance of all bees captured at each location (a set of traps, represented as “sample location” in the graph) during May (denoted as 1), June (2), July (3), August (4), September (5), October (6), and November (7) in 2011 (Left) and 2012 (Right). Practice: NM, NWSG mix with multiple biomass harvests (Practice code # 511); NS, NWSG mix with a single biomass harvest; SM, Switchgrass monoculture with multiple biomass harvests (Practice code # 511); SS, Switchgrass monoculture with a single biomass harvest.

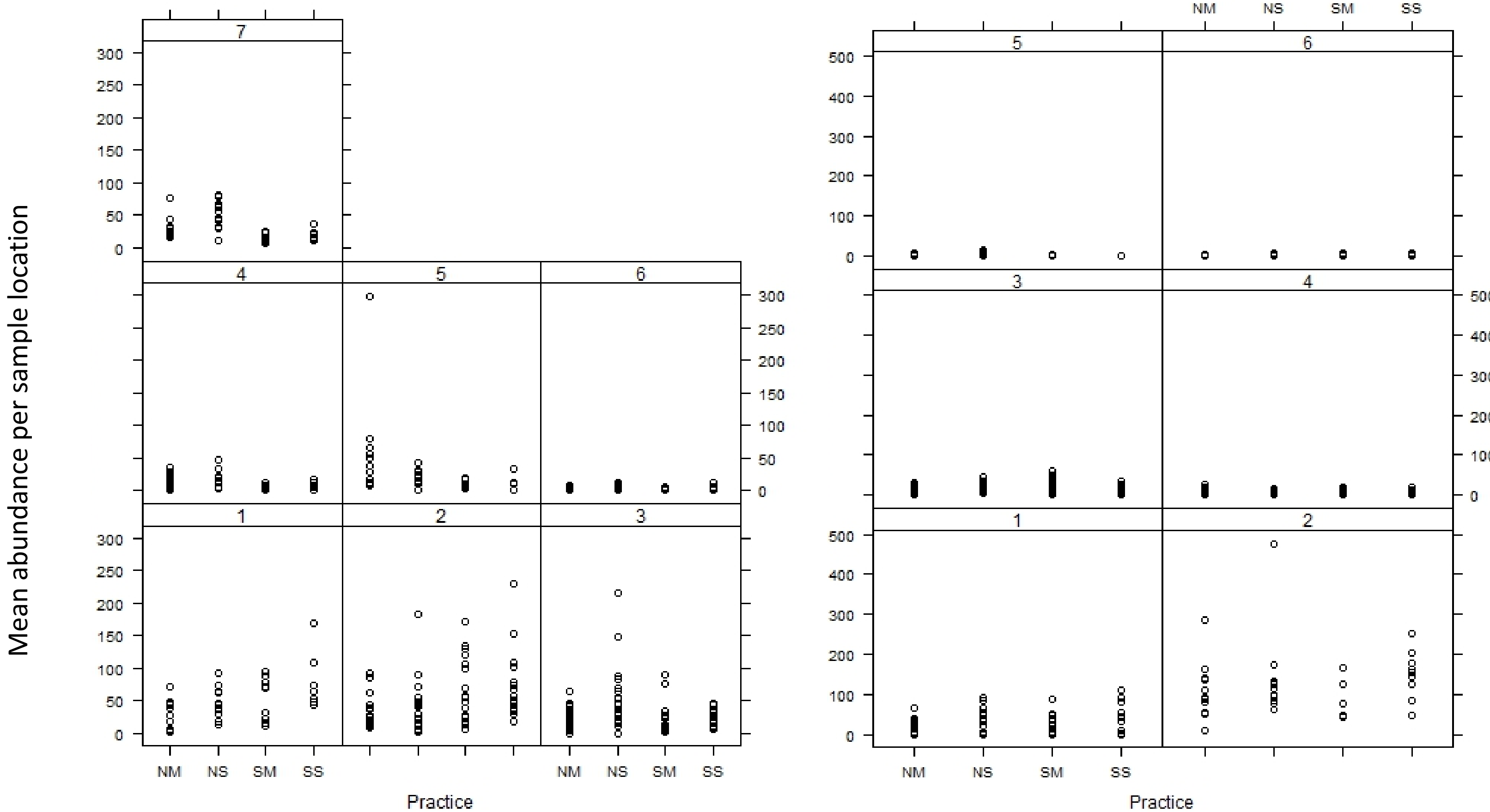



Figure 19. Mean abundance of ground-nesting bees captured at each location (a set of traps, represented as “sample location” in the graph) during May (denoted as 1), June (2), July (3), August (4), September (5), October (6), and November (7) in 2011 (Left) and 2012 (Right). Practice: NM, NWSG mix with multiple biomass harvests (Practice code # 511); NS, NWSG mix with a single biomass harvest; SM, Switchgrass monoculture with multiple biomass harvests (Practice code # 511); SS, Switchgrass monoculture with a single biomass harvest.

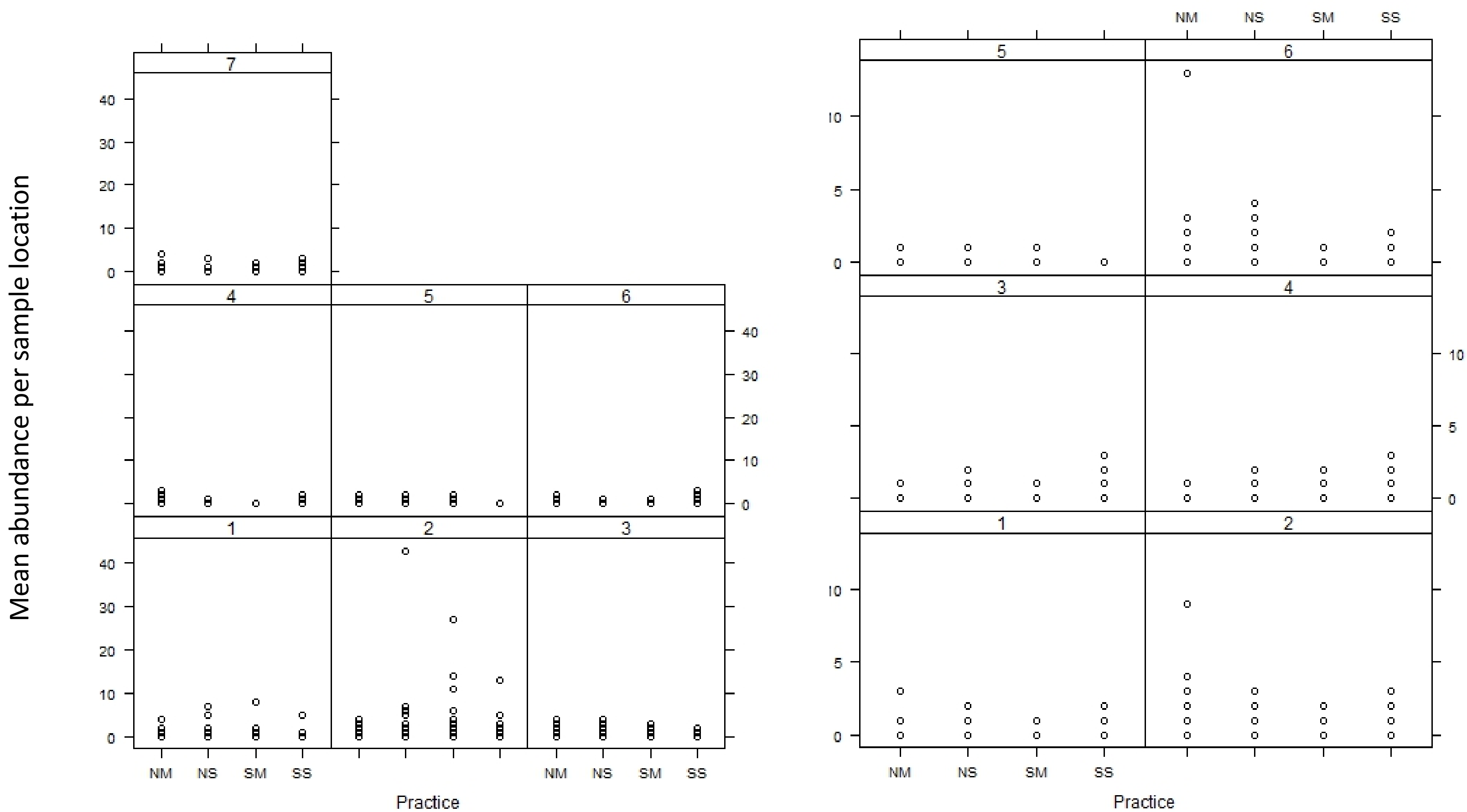



Figure 20. Mean abundance of non-ground nesting bees captured at each location (a set of traps, represented as “sample location” in the graph) during May (denoted as 1), June (2), July (3), August (4), September (5), October (6), and November (7) in 2011 (Left) and 2012 (Right). Practice: NM, NWSG mix with multiple biomass harvests (Practice code # 511); NS, NWSG mix with a single biomass harvest; SM, Switchgrass monoculture with multiple biomass harvests (Practice code # 511); SS, Switchgrass monoculture with a single biomass harvest.

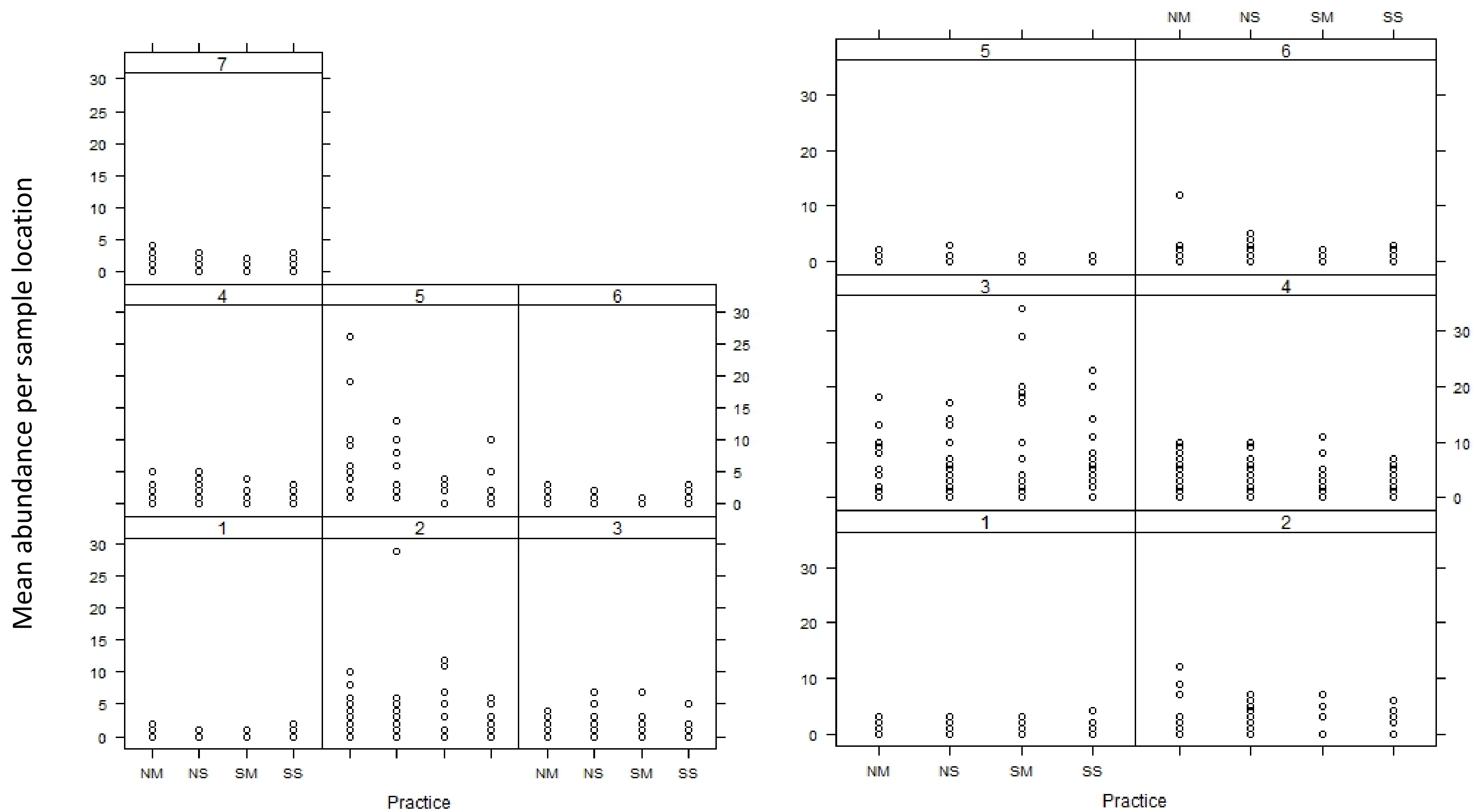
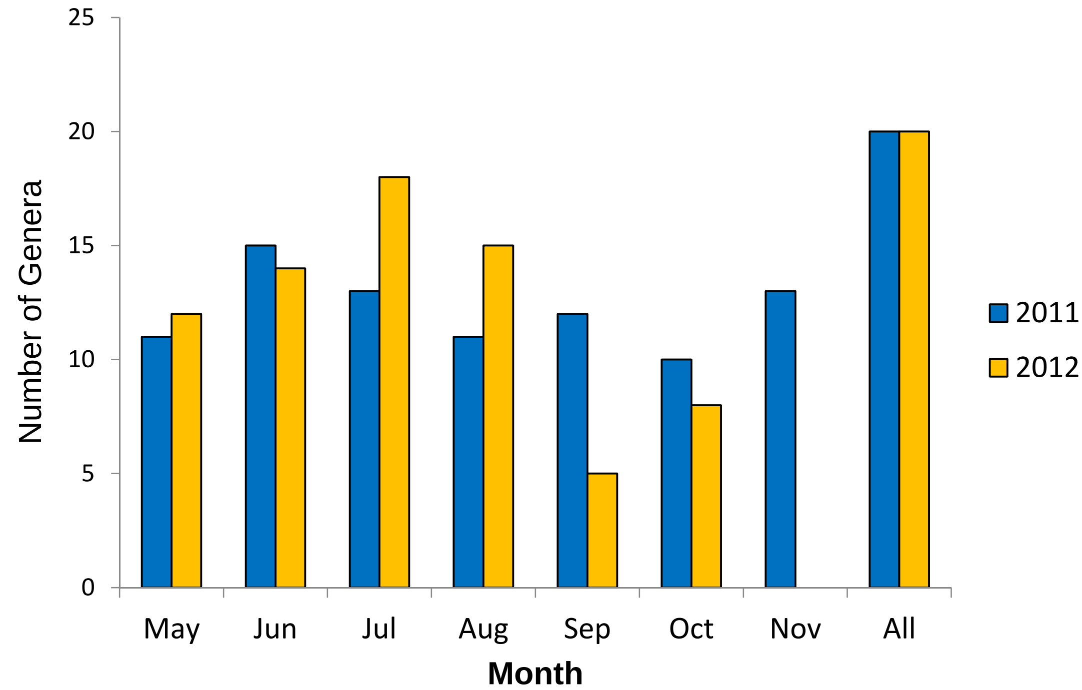



Figure 21. Mean abundance of bees of Apidae captured at each location (a set of traps, represented as "sample location" in the graph) during May (denoted as 1), June (2), July (3), August (4), September (5), October (6), and November (7) in 2011 (Left) and 2012 (Right). Practice: NM, NWSG mix with multiple biomass harvests (Practice code # 511); NS, NWSG mix with a single biomass harvest; SM, Switchgrass monoculture with multiple biomass harvests (Practice code # 511); SS, Switchgrass monoculture with a single biomass harvest.

Table 10. Summary of F-values (P-values in parenthesis) from a general linear mixed model with practice as fixed effect and field as random effect. Significant practice effects (P<0.10) are bolded.

| Year | Response variable (Abundance) | Month | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | May[1] | Jun[2] | Jul | Aug[1,3] | Sep[1,2] | Oct[2] | Nov[1] |
| 2011 | Total | 1.399 (0.300) | **5.096 (0.021)** | 2.648 (0.106) | 2.427 (0.126) | 2.626 (0.108) | 1.382 (0.304) | **11.957 (0.001)** |
| | Ground | 1.447 (0.287) | **5.657 (0.015)** | 2.252 (0.145) | 2.202 (0.151) | 2.518 (0.117) | 1.458 (0.284) | **12.235 (0.001)** |
| | Non-ground | 0.573 (0.646) | 0.508 (0.686) | 1.491 (0.276) | 1.435 (0.290) | 1.518 (0.270) | 0.925 (0.464) | 0.074 (0.973) |
| | Apidae | 0.615 (0.621) | 0.050 (0.984) | 0.572 (0.646) | **3.581 (0.054)** | 1.351 (0.313) | 1.685 (0.233) | 0.677 (0.586) |
| 2012 | Total | 0.870 (0.489) | 1.600 (0.612) | 1.546 (0.263) | 1.028 (0.421) | 1.273 (0.347) | 0.378 (0.771) | – |
| | Ground | 0.981 (0.440) | 0.662 (0.594) | 1.563 (0.259) | 1.304 (0.326) | 1.335 (0.330) | 1.250 (0.343) | – |
| | Non-ground | 0.084 (0.967) | 0.779 (0.532) | 0.998 (0.433) | 1.404 (0.298) | 0.20 (0.880) | 1.655 (0.239) | – |
| | Apidae | 0.413 (0.748) | 0.254 (0.857) | 0.998 (0.433) | 1.291 (0.330) | 0.511 (0.686) | 1.656 (0.239) | – |

[1] One trapping occasion in 2011
[2] One trapping occasion in 2012
[3] Three trapping occasions in 2012



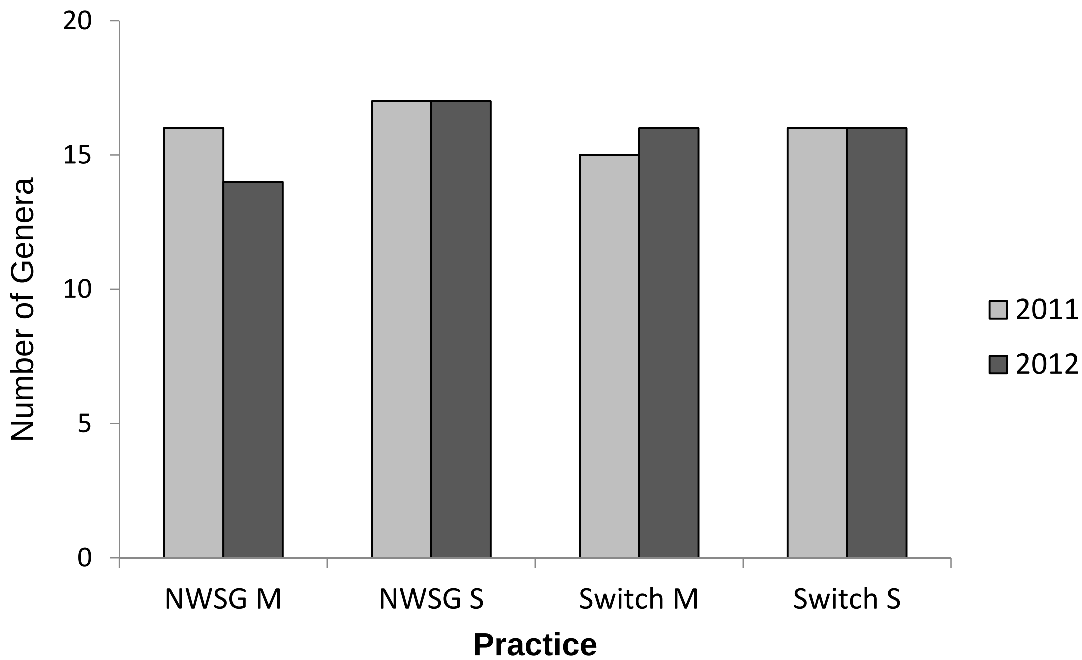




Figure 22. Genus richness (number of genera) of bees by month (upper) and practice (lower). "All" in the upper graph represents overall genus richness at each year, 2011 and 2012. Practice: NWSG M, NWSG mix with multiple biomass harvests (Practice code # 511); NWSG S, NWSG mix with a single biomass harvest; Switch M, Switchgrass monoculture with multiple biomass harvests (Practice code # 511); Switch S, Switchgrass monoculture with a single biomass harvest.

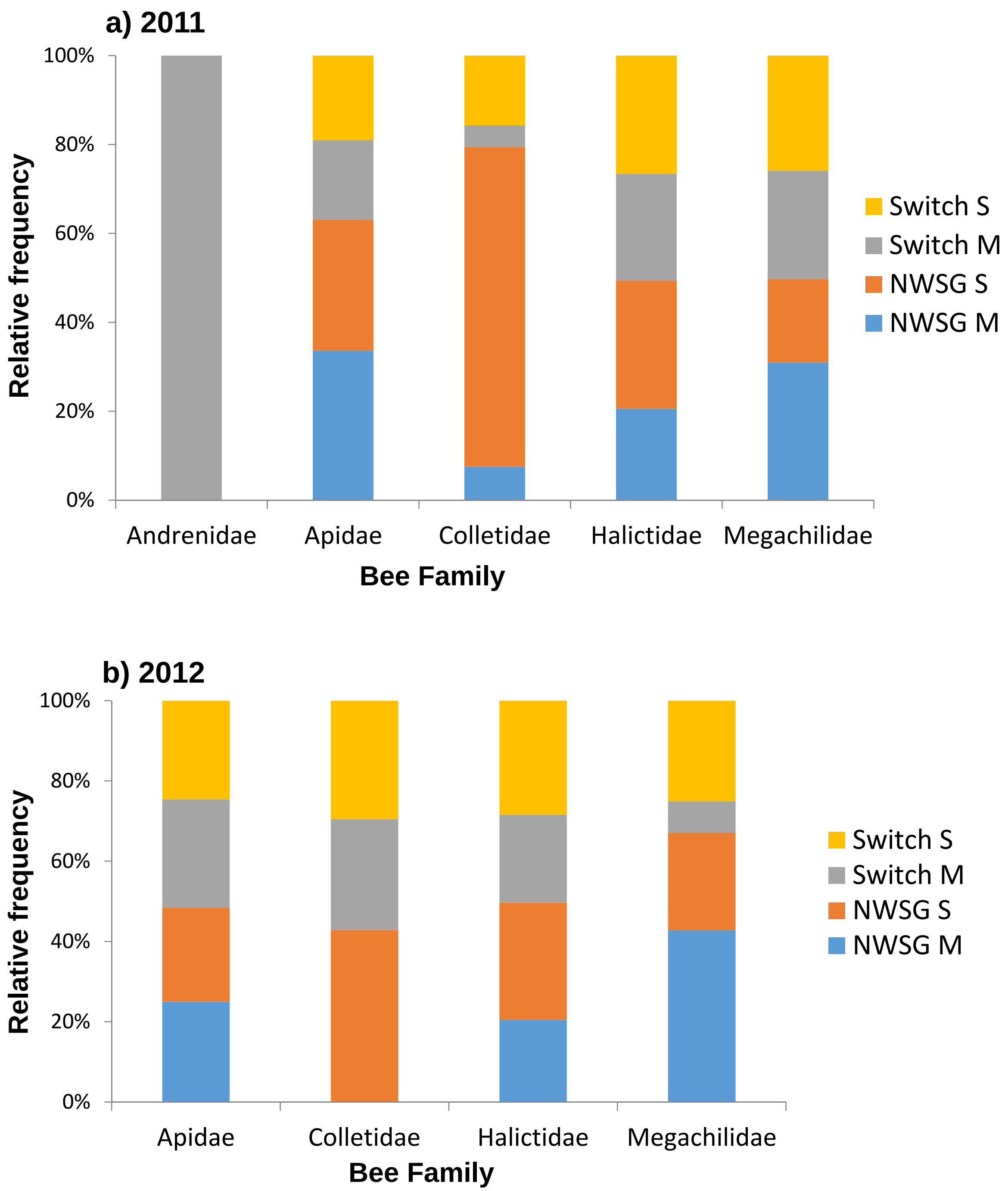
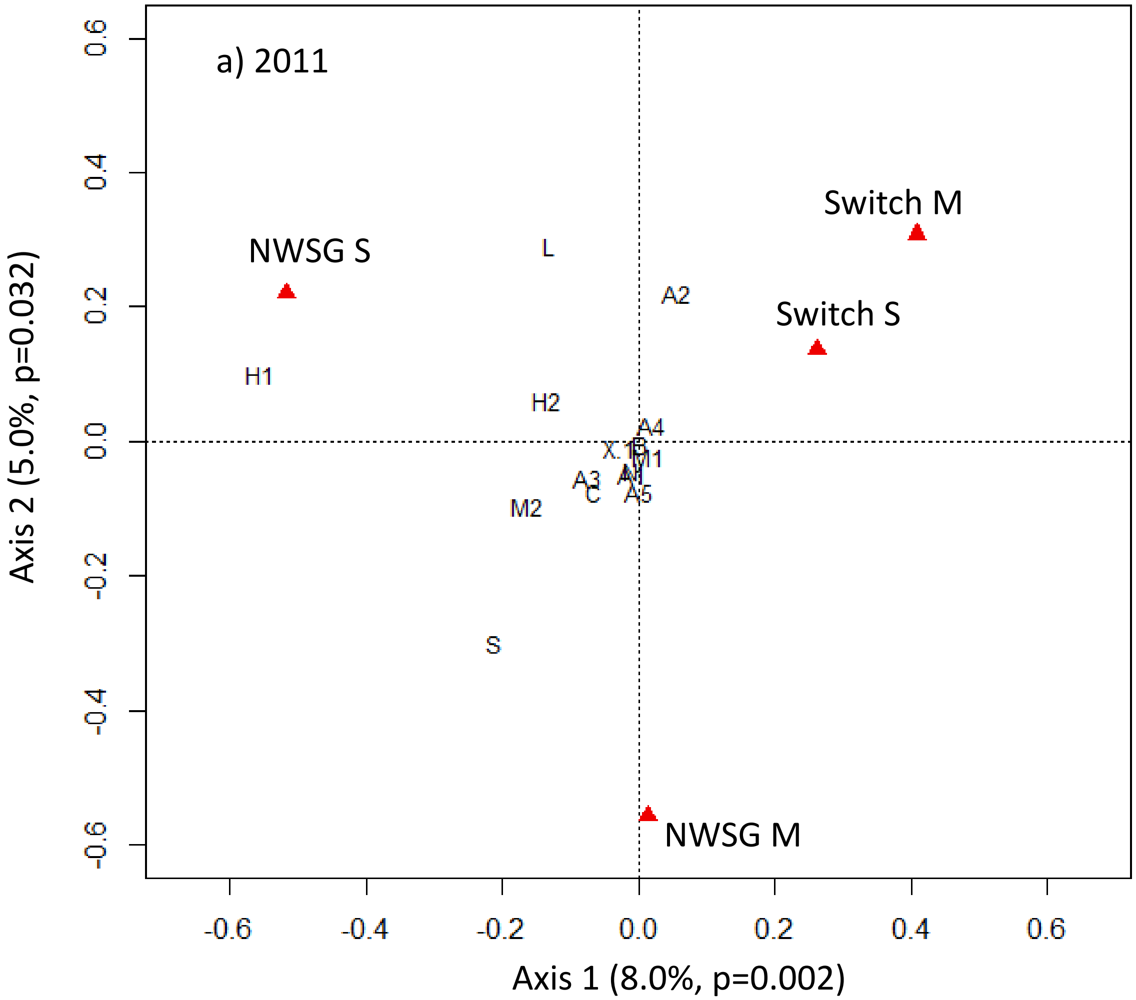



Figure 23. Relative proportion of abundance of five bee families at each practice. Note that only 4 individuals of Andrenidae were captured in 2011 and no individual was found in 2012. Practice: NWSG M, NWSG mix with multiple biomass harvests (Practice code # 511); NWSG S, NWSG mix with a single biomass harvest; Switch M, Switchgrass monoculture with multiple biomass harvests (Practice code # 511); Switch S, Switchgrass monoculture with a single biomass harvest.


a) 2011
NWSG S
L
A2
Switch M
Switch S
H1
H2
A4
X.1
B
M1
A3
AI
C
A5
M2
S
NWSG M
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6
Axis 2 (5.0%, p=0.032)
Axis 1 (8.0%, p=0.002)

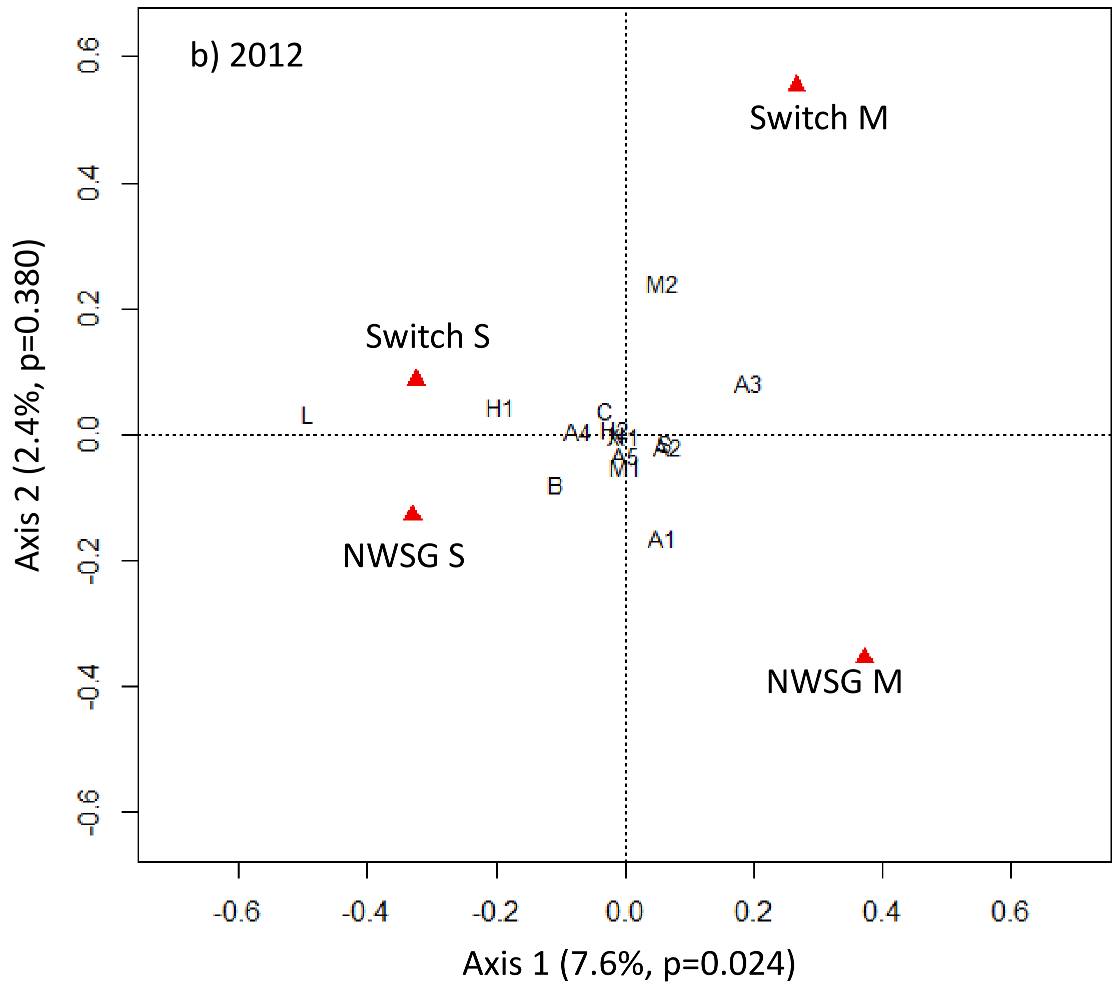



Figure 24. Redundancy analysis (RDA) biplot of four practices and bee genera in 2011(a) and 2012 (b). Variance (%) explained by each of the first two axis and *p* values calculated from permutation test of 999 iterations are shown in parenthesis. Practice: NWSG M, NWSG mix with multiple biomass harvests (Practice code # 511); NWSG S, NWSG mix with a single biomass harvest; Switch M, Switchgrass monoculture with multiple biomass harvests (Practice code # 511); Switch S, Switchgrass monoculture with a single biomass harvest. Genus: A1, Apis; A2, Augochlora; A3, Augochlorella; A4, Augochloropsis; A5, Agapostemon; B, Bombus; C, Certina; H1, Halictus; H2, Hylaeus; L, Lasioglossum; M1, Megachile; M2, Melissodes; N, Nomia; S, Svastra; X, Xylocopa.

Table 11. Order, family, and guild classification (based on foraging strategy) of all insects detected at Bryan Farms site, Mississippi during 2011-2012.

| **Order** | **Family** | **Foraging strategy** |
|---|---|---|
| Coleoptera | Buprestidae | Pollinators |
| | Cerambycidae | Herbivores |
| | Curculionidae | Herbivores |
| | Meloidae | Predators/parasites/parasitoids |
| | Mordellidae | Others/omnivores |
| | Scarabaeidae | Herbivores |
| Diptera | Bombyliidae | Pollinators, Predators/parasites/parasitoids |
| | Conopidae | Predators/parasites/parasitoids, Pollinators |
| | Dolichopodidae | Predators/parasites/parasitoids |
| | Stratiomyidae | Others/omnivores |
| | Syrphidae | Predators/parasites/parasitoids, Pollinators |
| Hymenoptera | Andrenidae | Pollinators |
| (Bees) | Apidae | Pollinators |
| | Colletidae | Pollinators |
| | Halictidae | Pollinators, Herbivores |
| | Megachilidae | Pollinators, Predators/parasites/parasitoids |
| Hymenoptera | Chrysididae | Predators/parasites/parasitoids |
| (Non-bees, | Crabronidae | Predators/parasites/parasitoids |
| Wasps) | Scoliidae | Pollinators |
| | Sphecidae | Predators/parasites/parasitoids |
| | Tiphiidae | Pollinators |
| | Vespidae | Predators/parasites/parasitoids |

| | | |
|---|---|---|
| Lepidoptera | Hesperiidae | Pollinators, Herbivores |
| | Lycaenidae | Pollinators |
| | Nymphalidae | Pollinators |
| | Papilionidae | Pollinators |
| | Pieridae | Pollinators |
| | Sphingidae | Pollinators |

Table 12. Number of individuals of each family of insects detected at Bryan Farms site, Mississippi during 2011-2012.

| **Family** | **2011** | | | | | | | | **2012** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | May[1] | Jun | Jul | Aug[1] | Sep[1] | Oct | Nov[1] | **TOTAL** | May | Jun[1] | Jul | Aug[2] | Sep[1] | Oct[1] | **TOTAL** |
| Andrenidae | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Apidae | 19 | 211 | 103 | 72 | 189 | 37 | 39 | 670 | 67 | 134 | 500 | 325 | 19 | 69 | 1114 |
| Bombyliidae | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 0 | 1 | 0 | 0 | 0 | 4 |
| Buprestidae | 18 | 19 | 0 | 0 | 0 | 0 | 0 | 37 | 12 | 14 | 1 | 0 | 0 | 0 | 27 |
| Cerambycidae | 0 | 0 | 1 | 1 | 0 | 0 | 2 | 4 | 0 | 3 | 1 | 0 | 5 | 0 | 9 |
| Chrysididae | 0 | 2 | 1 | 0 | 0 | 0 | 1 | 4 | 0 | 0 | 1 | 1 | 0 | 0 | 2 |
| Colletidae | 12 | 7 | 0 | 0 | 0 | 5 | 1 | 25 | 0 | 0 | 1 | 1 | 0 | 2 | 4 |
| Conopidae | 0 | 3 | 0 | 1 | 1 | 1 | 3 | 9 | 2 | 17 | 1 | 0 | 0 | 0 | 20 |
| Crabronidae | 2 | 4 | 1 | 0 | 2 | 0 | 0 | 9 | 10 | 18 | 12 | 4 | 1 | 0 | 45 |
| Curculionidae | 0 | 0 | 0 | 3 | 6 | 6 | 0 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dolichopodidae | 368 | 562 | 2127 | 1829 | 5848 | 11684 | 1207 | 23625 | 6295 | 8105 | 2064 | 6829 | 2743 | 2206 | 28242 |
| Halictidae | 1986 | 3810 | 2404 | 419 | 909 | 226 | 1242 | 10996 | 1880 | 5240 | 970 | 499 | 72 | 57 | 8718 |
| Hesperiidae | 4 | 27 | 15 | 24 | 7 | 46 | 31 | 154 | 11 | 17 | 19 | 90 | 5 | 85 | 227 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lycaenidae | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 3 | 0 | 0 | 0 | 0 | 4 |
| Megachilidae | 0 | 2 | 3 | 0 | 1 | 4 | 4 | 14 | 3 | 2 | 1 | 1 | 1 | 7 | 15 |
| Meloidae | 0 | 3 | 12 | 14 | 4 | 13 | 1 | 47 | 6 | 10 | 11 | 14 | 12 | 6 | 59 |
| Mordellidae | 11 | 149 | 46 | 13 | 21 | 13 | 6 | 259 | 106 | 194 | 66 | 9 | 3 | 4 | 382 |
| Nymphalidae | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Papilonidae | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 6 |
| Pieridae | 2 | 3 | 2 | 0 | 0 | 2 | 2 | 11 | 2 | 6 | 5 | 13 | 0 | 0 | 26 |
| Scarabaeidae | 2 | 1 | 0 | 0 | 2 | 2 | 0 | 7 | 0 | 1 | 0 | 2 | 0 | 0 | 3 |
| Scoliidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 2 |
| Sphecidae | 50 | 24 | 0 | 0 | 0 | 1 | 1 | 76 | 0 | 11 | 4 | 6 | 0 | 0 | 21 |
| Sphingidae | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Stratiomyidae | 0 | 0 | 1 | 1 | 6 | 3 | 0 | 11 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Syrphidae | 1516 | 370 | 93 | 58 | 149 | 298 | 992 | 3476 | 333 | 171 | 17 | 38 | 23 | 84 | 666 |
| Tiphiidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 0 | 0 | 6 |
| Vespidae | 0 | 8 | 21 | 5 | 43 | 28 | 12 | 117 | 5 | 17 | 22 | 9 | 0 | 6 | 59 |
| **TOTAL** | 3994 | 5206 | 4830 | 2440 | 7188 | 12371 | 3546 | 39575 | 8740 | 13967 | 3701 | 7844 | 2884 | 2526 | 39662 |

[1]Surveyed only once
[2]Surveyed three times

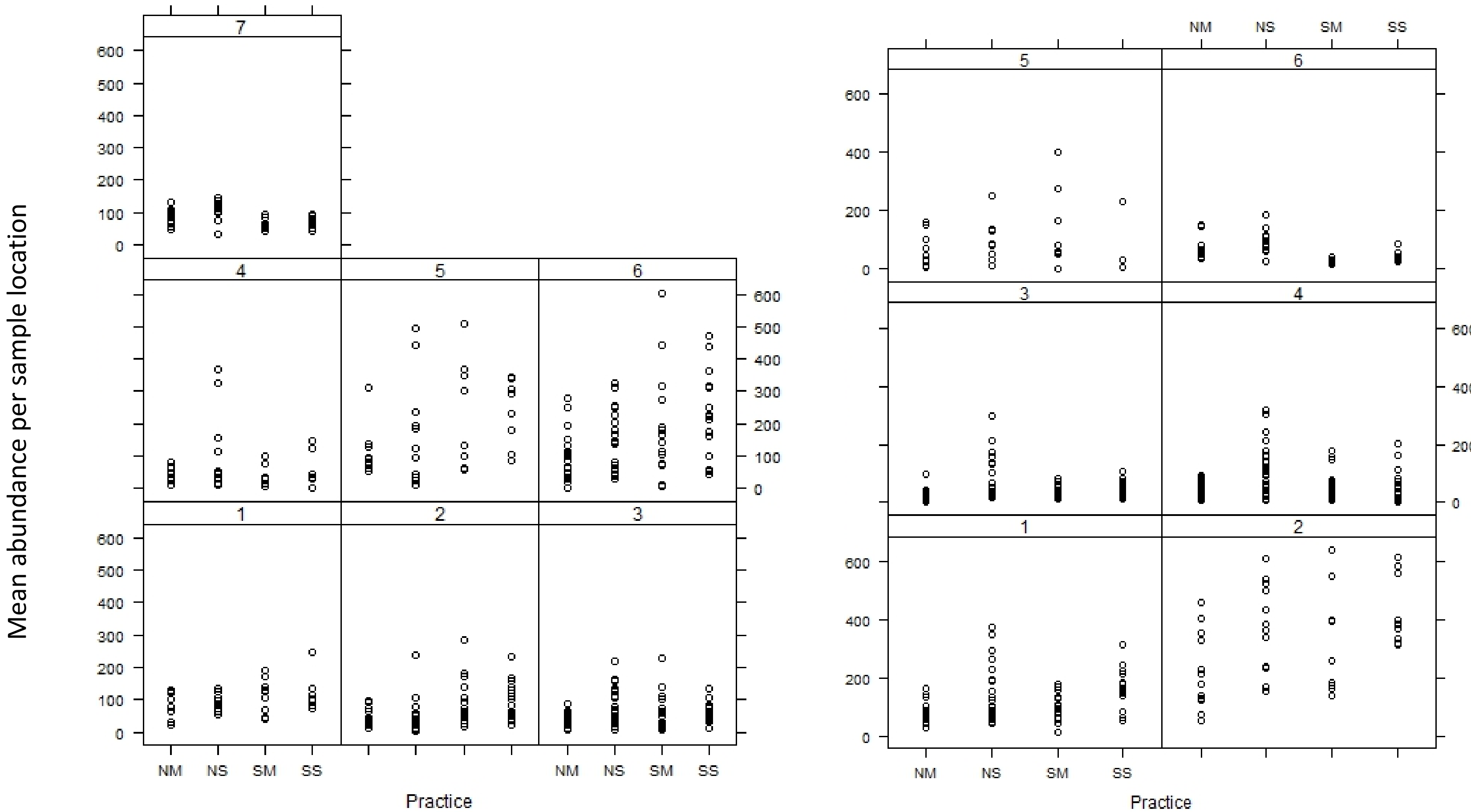



Figure 25. Mean abundance of all insects captured at each location (a set of traps, represented as "sample location" in the graph) during May (denoted as 1), June (2), July (3), August (4), September (5), October (6), and November (7) in 2011 (Left) and 2012 (Right). Practice: NM, NWSG mix with multiple biomass harvests (Practice code # 511); NS, NWSG mix with a single biomass harvest; SM, Switchgrass monoculture with multiple biomass harvests (Practice code # 511); SS, Switchgrass monoculture with a single biomass harvest.

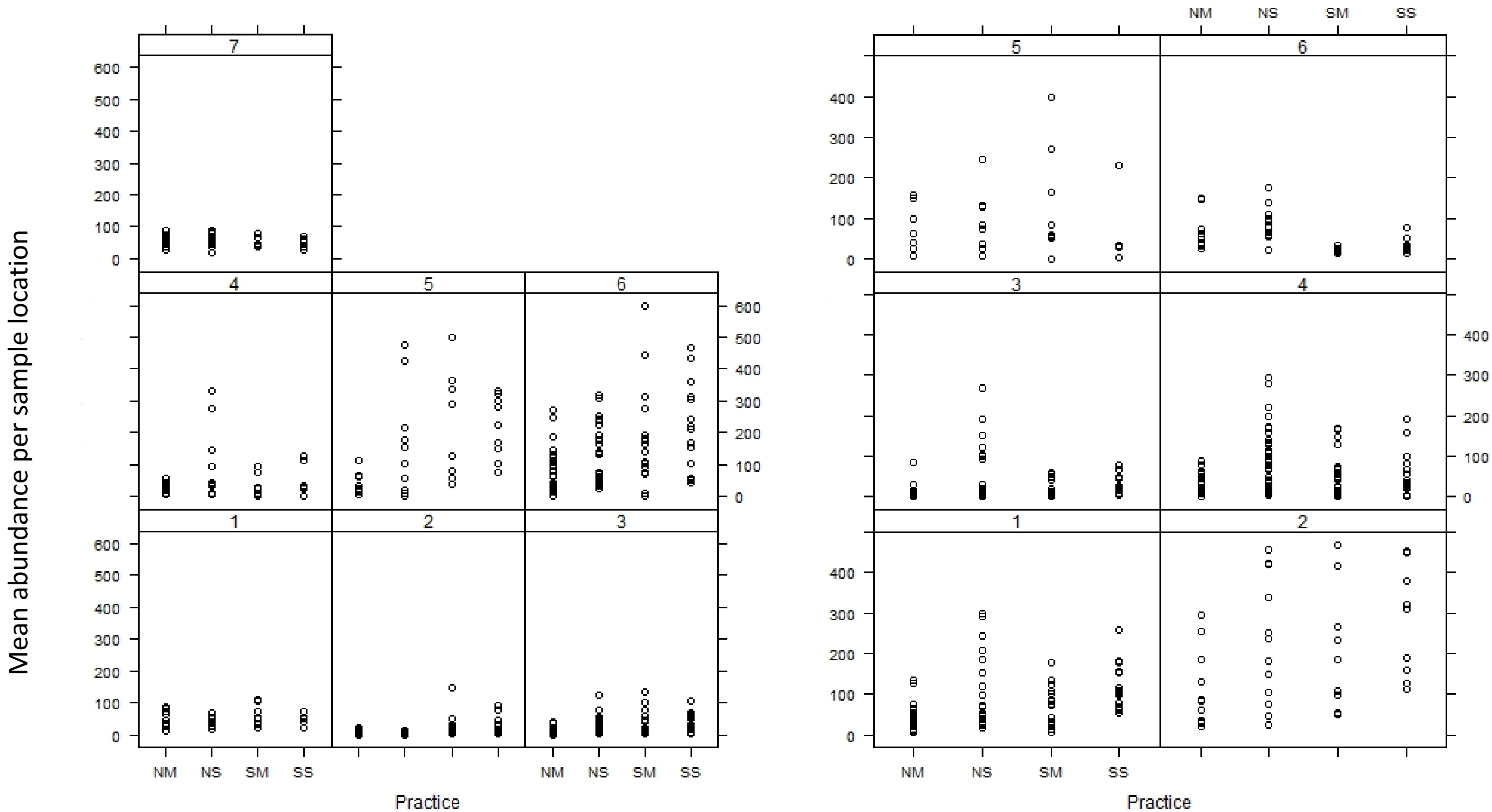



Figure 26. Mean abundance of predator/parasite insects captured at each location (a set of traps, represented as "sample location" in the graph) during May (denoted as 1), June (2), July (3), August (4), September (5), October (6), and November (7) in 2011 (Left) and 2012 (Right). Practice: NM, NWSG mix with multiple biomass harvests (Practice code # 511); NS, NWSG mix with a single biomass harvest; SM, Switchgrass monoculture with multiple biomass harvests (Practice code # 511); SS, Switchgrass monoculture with a single biomass harvest.

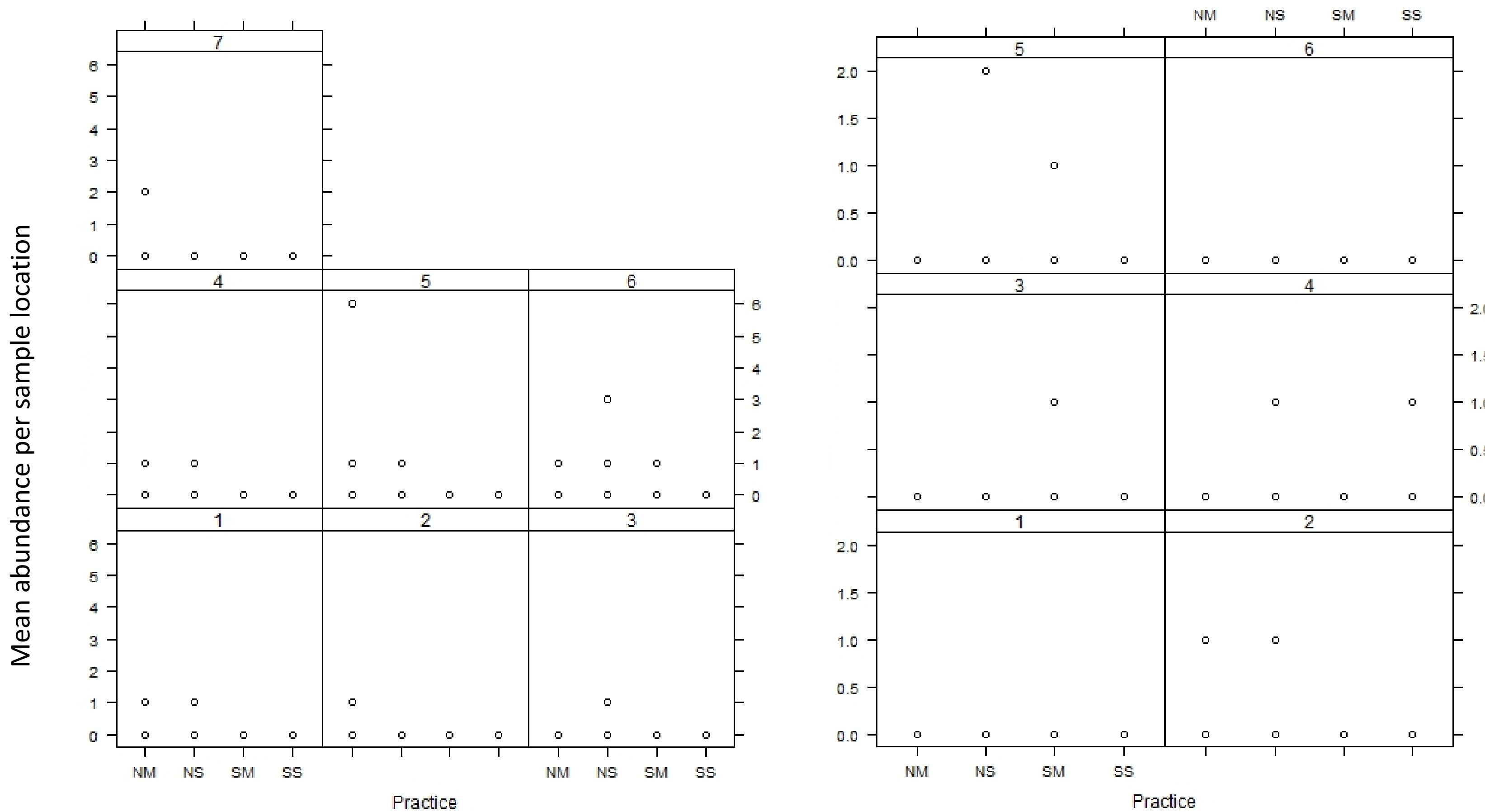



Figure 27. Mean abundance of herbivore insects captured at each location (a set of traps, represented as “sample location” in the graph) during May (denoted as 1), June (2), July (3), August (4), September (5), October (6), and November (7) in 2011 (Left) and 2012 (Right). Practice: NM, NWSG mix with multiple biomass harvests (Practice code # 511); NS, NWSG mix with a single biomass harvest; SM, Switchgrass monoculture with multiple biomass harvests (Practice code # 511); SS, Switchgrass monoculture with a single biomass harvest.

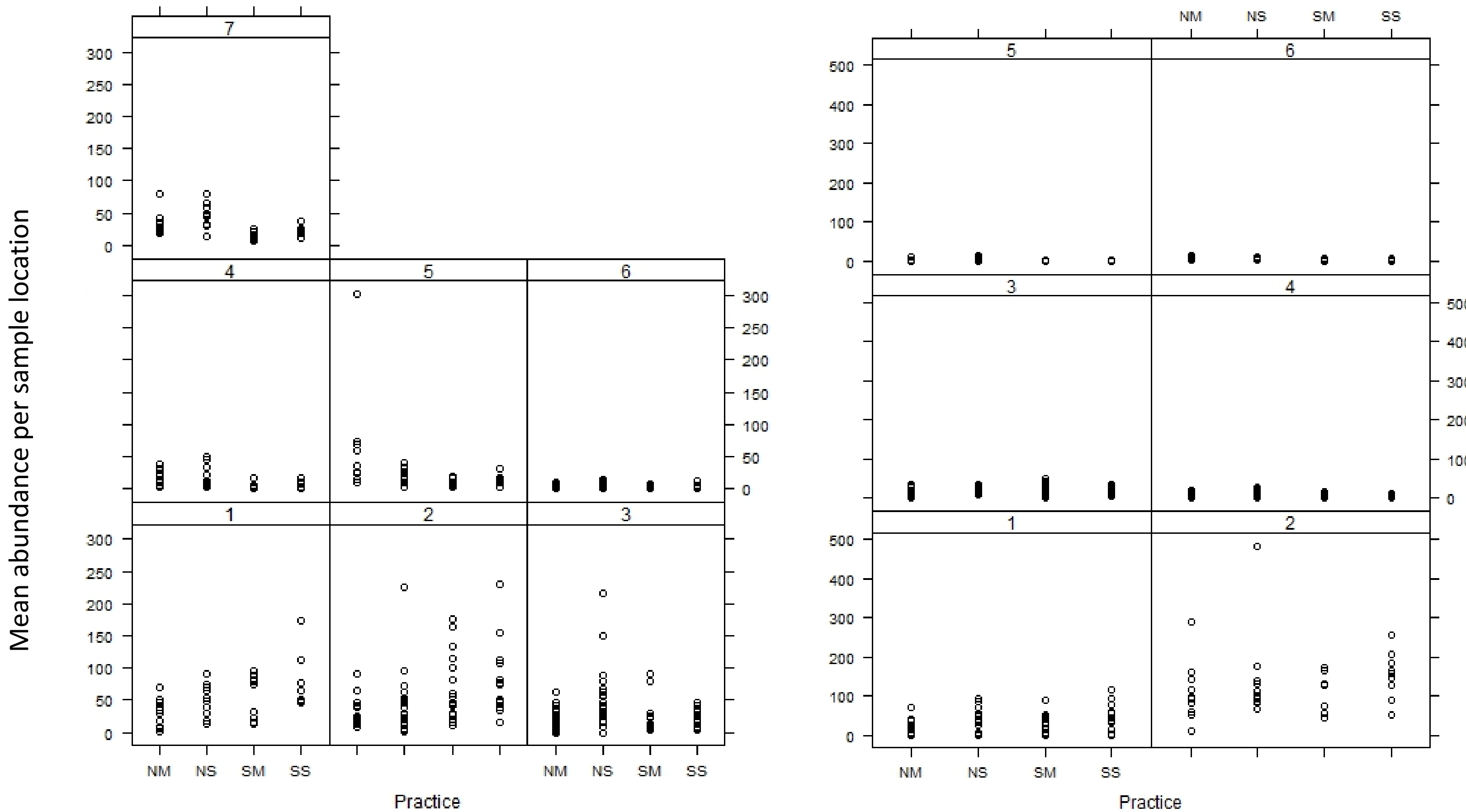
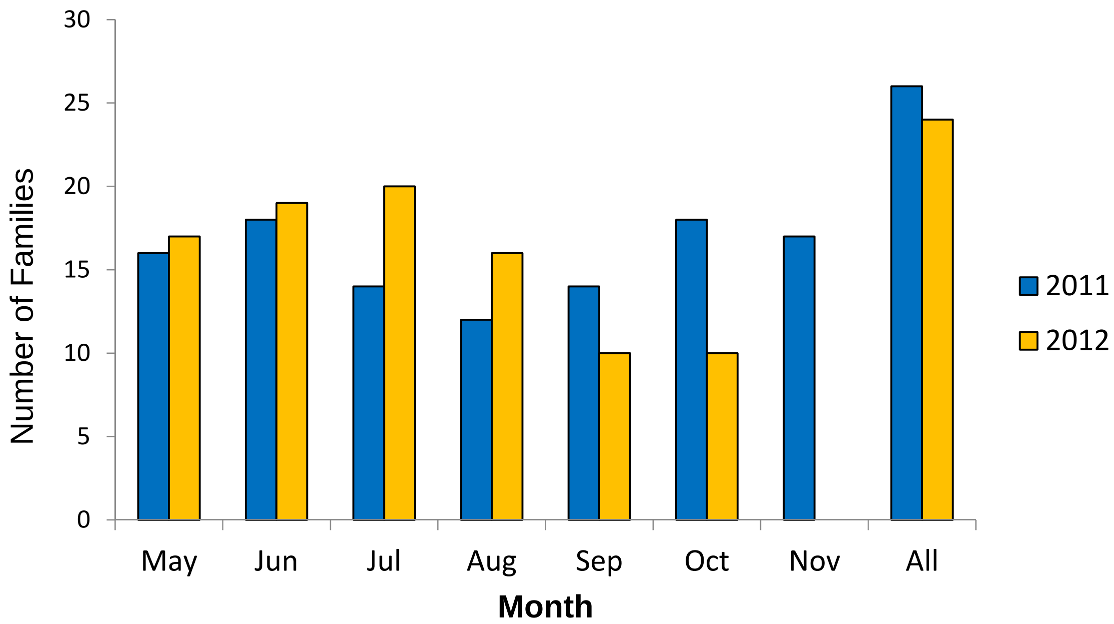



Figure 28. Mean abundance of pollinator insects captured at each location (a set of traps, represented as “sample location” in the graph) during May (denoted as 1), June (2), July (3), August (4), September (5), October (6), and November (7) in 2011 (Left) and 2012 (Right). Practice: NM, NWSG mix with multiple biomass harvests (Practice code # 511); NS, NWSG mix with a single biomass harvest; SM, Switchgrass monoculture with multiple biomass harvests (Practice code # 511); SS, Switchgrass monoculture with a single biomass harvest.



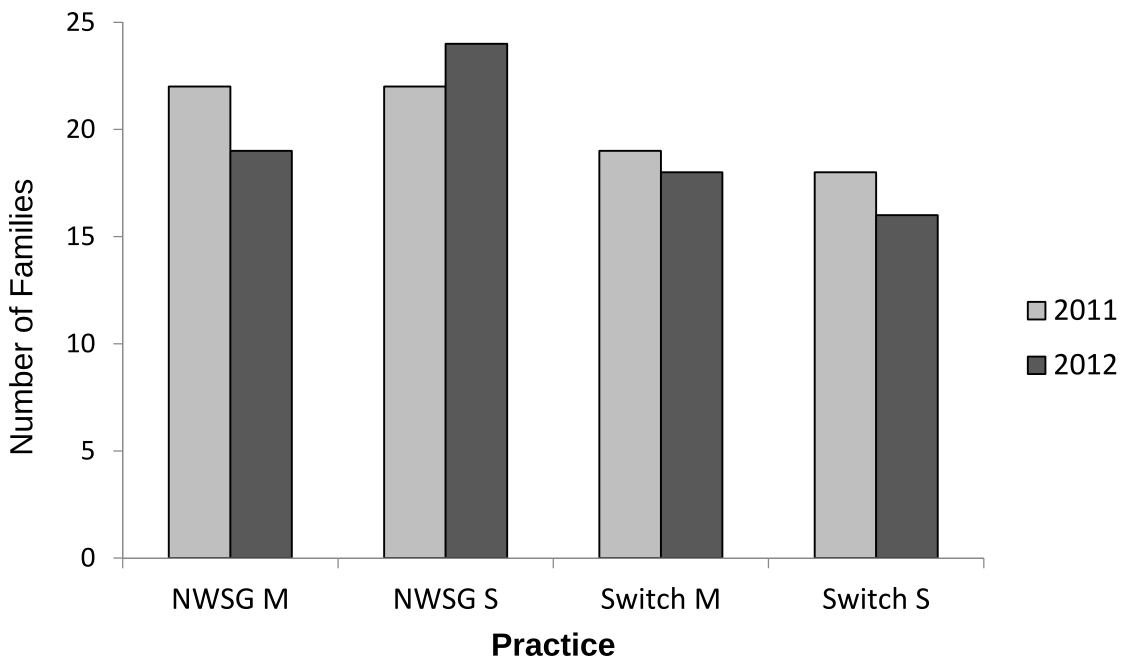




Figure 29. Family richness (number of families) of insects by month (upper) and practice (lower). “All” in the upper graph represents overall family-level richness at each year, 2011 and 2012. Practice: NWSG M, NWSG mix with multiple biomass harvests (Practice code # 511); NWSG S, NWSG mix with a single biomass harvest; Switch M, Switchgrass monoculture with multiple biomass harvests (Practice code # 511); Switch S, Switchgrass monoculture with a single biomass harvest.

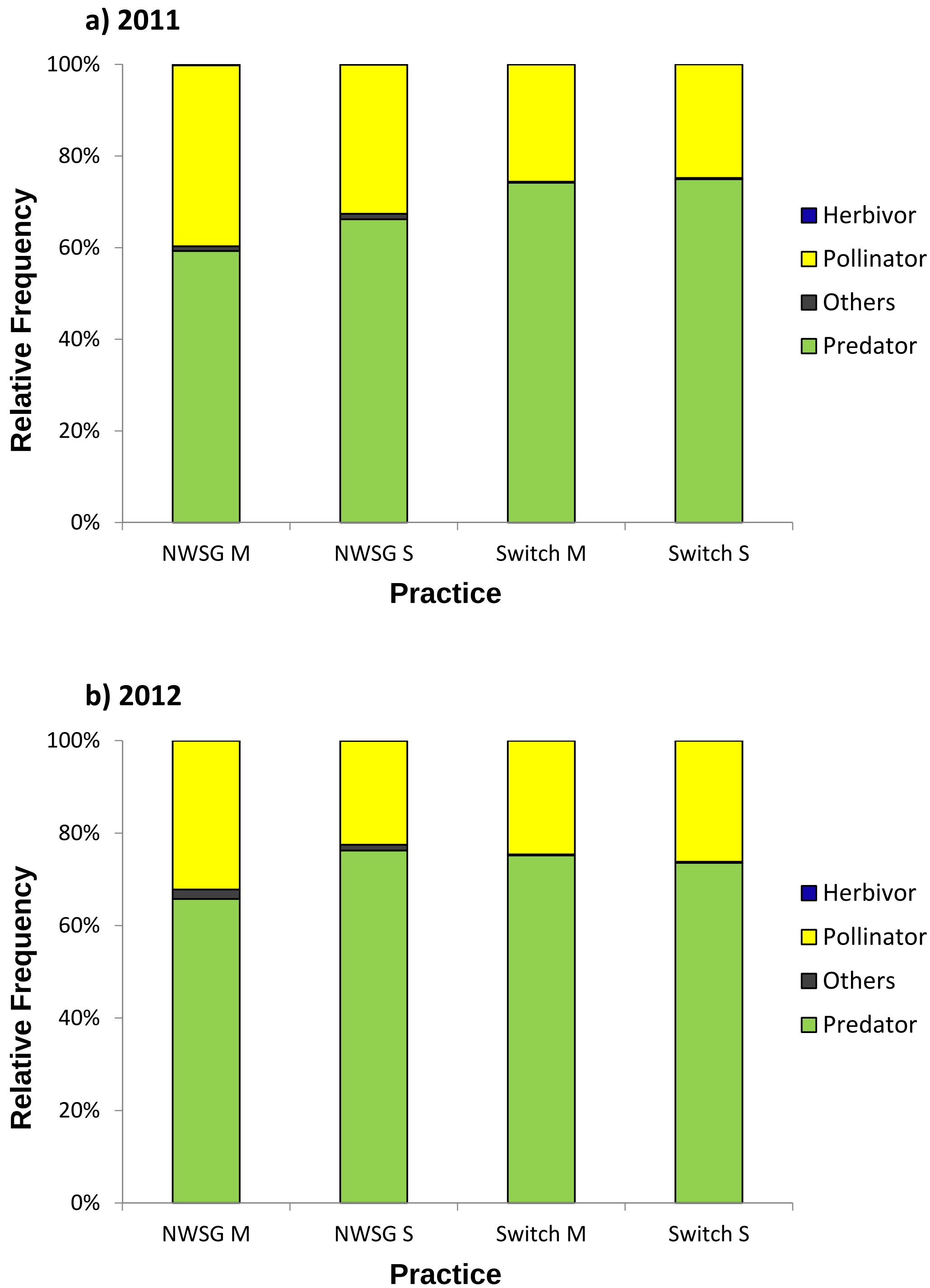



Figure 30. Relative proportion of abundance of insect foraging guilds at each practice. "Predator" represents predators/parasites/parasitoids foraging guild. Practice: NWSG M, NWSG mix with multiple biomass harvests (Practice code # 511); NWSG S, NWSG mix with a single biomass harvest; Switch M, Switchgrass monoculture with multiple biomass harvests (Practice code # 511); Switch S, Switchgrass monoculture with a single biomass harvest.

## 3. Scooba Site

### 3.1. Bees

#### 3.1.1. General pattern

A total of 2511 bees (18 genera) were trapped during 2013-2014 (Table 13 and Table 14): 1002 bees in 2011 and 1509 bees in 2012. Lasioglossum and Ceratina were the most abundant, comprising 72% of total bees in 2013. Lasioglossum, Ceratina, and Augochlorella accounted for 87% of total bees in 2014. Abundance of bees belonging to Apidae was 41% of total bees in 2013 and 25% in 2014. Like the seasonal patterns found in other sites, abundance of bee was higher in June both years.

#### 3.1.2. Effect of practice

*Abundance*

Mean abundance of bees (total, both nesting guilds and Apidae) varied by month, year, and practices (Fig. 31-34). In 2011, mean abundance of ground nesting bees was high at 5-year old stand (PI and SI) during May-August, whereas non-ground nesting bees and Apidae was more abundant at 10-year old stand (T-PI and T-PISI). Mean abundance of total bees tended to be higher at 10-year old stand in June and at 5-year old stand in July. On the other hand, in 2014, total bees and ground nesting bees were more abundant at PI and SI. Non-ground nesting bees and Apidae did not show clear patterns although their abundance was higher at T-PI in June.

Based on the results of the generalized mixed model, significant or week practice effect was found in abundance of non-ground nesting guilds and Apidae in 2013 (Table 15). Practice also influenced abundance of total bees and ground nesting bees in 2014.

*Genus richness*

Although genus richness in June and July was greater in 2014 than in 2013, overall richness was greater in 2013 (Fig. 35). Effect of practice on genus richness was also varied by year: Greater richness was observed at T-PISI in 2013 and SI in 2014. Richness was low at T-PI both years.

*Composition of bee community*

At family level, Apidae was found more at 10-years old stand in 2013, but at 5-years old stand in 2014 (Fig. 36). Colletidae, Halictidae and Megachilidae showed similar pattern between years: more abundant at 5-year old stand.

At genus level, genus composition among four practices was significantly different and the dissimilarity was relatively strong compared to other two sites: ANOSIM statistic $R = 0.446$, $p = 0.001$ in 2013 and ANOSIM statistic $R = 0.415$, $p = 0.001$ in 2014. Similarly, two canonical axes from RDA explained 44% (2013) and 56% (2014) of variations in the data. In particular, Axis 1 that represents a gradient between 5- year old stand and 10-year old stand explained

41.5% (2013) and 53% (2014) of the variations, suggesting the stronger influence of stand age on bee community composition than practice of switchgrass intercropping (Fig. 37). Lasioglossum (L) was positively related to 5- year old stand and Augochlora (A2), Augochlorella (A3) was more associated with 10-year old stand in 2013. The positive association between A3 and 10-year old stand was also observed in 2014. Certina (C) and Melissode (M2) were positively related to 5-year old stand.

3.2. Insects

3.2.1. General pattern

A total of 15571 individual insects of 29 families were trapped during 2013-2014 (Table 16 and Table 17). Although there was one more trapping occasion in 2014, total number of insects captured was higher in 2013: 8604 in 2013 and 6967 in 2014. Insect was more abundant during June both years. Among 29 families, Dolichopodidae and Cicadellidae were the most abundant, comprising 61.3% (2013) and 57.8% (2014) of total insects.

3.2.2. Effect of practice

*Abundance*

Although mean abundance of insects per location was varied within a practice, there were also significant variations between practices, particularly between 5-year old stand (PI and SI) and 10-year old stand (T-PI and T-PISI) (Fig. 38-41). Mean abundance of total insects and predator/parasite/parasitoid insects was high at 5-year old stand both years. While pollinators also showed similar pattern during July-August in 2014, abundance of pollinators was low at those stands during June in 2013. Abundance of herbivore insects was also higher at 5-year old stand both years. Unlike this strong effect of stand age on insect abundance, effect of switchgrass intercropping was not clear given mean abundance was similar within the same aged-stands (e.g., PI and SI, T-PI and T-PISI). However, total insects tended to be slightly more abundant at PI than SI both years and at T-PISI than T- PI (except August), indicating that the effect of switchgrass intercropping might vary with sand age.

*Family richness*

Family richness was higher in 2013 than in 2014 except May (Fig. 42). Among four practices, greater family richness was observed at PI in 2013 and pine stands with intercropped switchgrass (SI and T-PISI) in 2014.

*Composition of insect community*

In 2013, predators/parasites/parasitoids were dominant at 5-year old stand whereas both predators/parasites/parasitoids and pollinators were dominant at 10-year old stand (Fig. 43, a). In 2014, similar pattern was found at 10-year old stand (Fig. 43, b). Compared to 2013, relative proportion of pollinators increased at all stands, particularly at 5-year old stand in 2014.

Table 13. Family, genus (and species), and guild classification (based on nest placement) of bees detected during 2013-2014, at Scooba site, Mississippi.

| **Family** | **Genus** | **Species** | **Nest placement guild** |
|---|---|---|---|
| Andrenidae | Andrena | | Ground nesting |
| | | *3Unknown Andrena species* | |
| | Perdita | | Ground nesting |
| | | *Perdita boltoniae* | |
| Apidae | Apis | | Eusocial hive[a] |
| | | *Apis mellifera* | |
| | Bombus | | Ground nesting |
| | | *Bombus griseocollis* | |
| | | *Bombus pennsylvanicus* | |
| | | *Bombus bimaculatus* | |
| | | *Bombus impatiens* | |
| | | *Bombus fraternus* | |
| | Ceratina | | Wood/Stem nesting |
| | | *Ceratina strenua* | |
| | Melissodes | | Ground nesting |
| | | *Melissodes agilis* | |
| | | *Melissodes communis* | |
| | | *Melissodes comptoides* | |
| | | *Melissodes bimaculata* | |
| | Melitoma | | Ground nesting |
| | | *Melitoma taurea* | |
| | Svastra | | Ground nesting |
| | | *Svastra atripes* | |
| | Xylocopa | | Wood nesting |

| | | | |
|---|---|---|---|
| | | *Xylocopa mican* | |
| Colletidae | Hylaeus | | Stem nesting |
| | | *Hylaeus mesillae* | |
| | | *Hylaeus modestus* | |
| | | *Hylaeus ornatus* | |
| Halictidae | Augochlorella | | Ground nesting |
| | | *Augochlorella aurata* | |
| | | *4Unknown Augochlorella species* | |
| | Augochlora | | Wood nesting |
| | | *Augochlora pura* | |
| | Halictus | | Ground nesting |
| | | *Halictus poeyi* | |
| | Lasioglossum | | Ground nesting |
| | | *Lasioglossum Zephyrium* | |
| | | *4Unknown Lasioglossum species* | |
| Megachilidae | Coelioxys | | Wood/Stem nesting |
| | | *Coelioxys octodentata/sayi* | |
| | Hoplitis | | Wood/Stem nesting |
| | | *Hoplitis simplex* | |
| | Megachile | | |
| | | *Megachile albitarsis* | |
| | | *Megachile brevis* | |
| | | *Megachile rotundata* | |
| | Osmia | | Wood/Stem nesting |
| | | *Osmia georgica* | |

[a] Considered as non- ground nesting for analysis.

Table 14. Number of individuals of each of bee genera detected at Scooba site, Mississippi during 2013-2014.

| **Genus** | **2013** | | | | | **2014** | | | | **Total** |
|---|---|---|---|---|---|---|---|---|---|---|
| | May[1] | Jun | Jul | Aug | | May[1] | Jun[2] | Jul | Aug | |
| Andrena | 3 | 0 | 1 | 0 | | 1 | 0 | 0 | 0 | 5 |
| Apis | 6 | 3 | 1 | 0 | | 1 | 2 | 0 | 0 | 13 |
| Augochlora | 5 | 11 | 15 | 23 | | 2 | 4 | 3 | 7 | 70 |
| Augochlorella | 9 | 21 | 20 | 35 | | 1 | 101 | 26 | 115 | 328 |
| Bombus | 4 | 12 | 8 | 3 | | 1 | 23 | 10 | 4 | 65 |
| Ceratina | 25 | 207 | 32 | 22 | | 13 | 151 | 58 | 10 | 518 |
| Coelixys | 0 | 0 | 0 | 0 | | 0 | 1 | 0 | 0 | 1 |
| Halictus | 0 | 1 | 0 | 0 | | 1 | 3 | 1 | 0 | 6 |
| Hoplotis | 1 | 0 | 0 | 0 | | 0 | 5 | 1 | 0 | 7 |
| Hylaeus | 1 | 8 | 1 | 0 | | 0 | 5 | 1 | 6 | 22 |
| Lasioglossum | 65 | 165 | 96 | 106 | | 15 | 320 | 180 | 323 | 1270 |
| Megachile | 0 | 0 | 0 | 1 | | 0 | 2 | 3 | 0 | 6 |
| Melissodes | 1 | 3 | 73 | 7 | | 1 | 5 | 43 | 53 | 186 |
| Melitoma | 0 | 1 | 0 | 0 | | 0 | 1 | 0 | 0 | 2 |
| Osmia | 1 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 1 |
| Perdita | 2 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 2 |
| Svastra | 0 | 0 | 0 | 1 | | 0 | 0 | 1 | 0 | 2 |
| Xylocopa | 1 | 0 | 1 | 0 | | 0 | 4 | 1 | 0 | 7 |
| **Total** | 124 | 432 | 248 | 198 | | 36 | 627 | 328 | 518 | 2511 |

[1] One sampling occasion
[2] Three sampling occasions

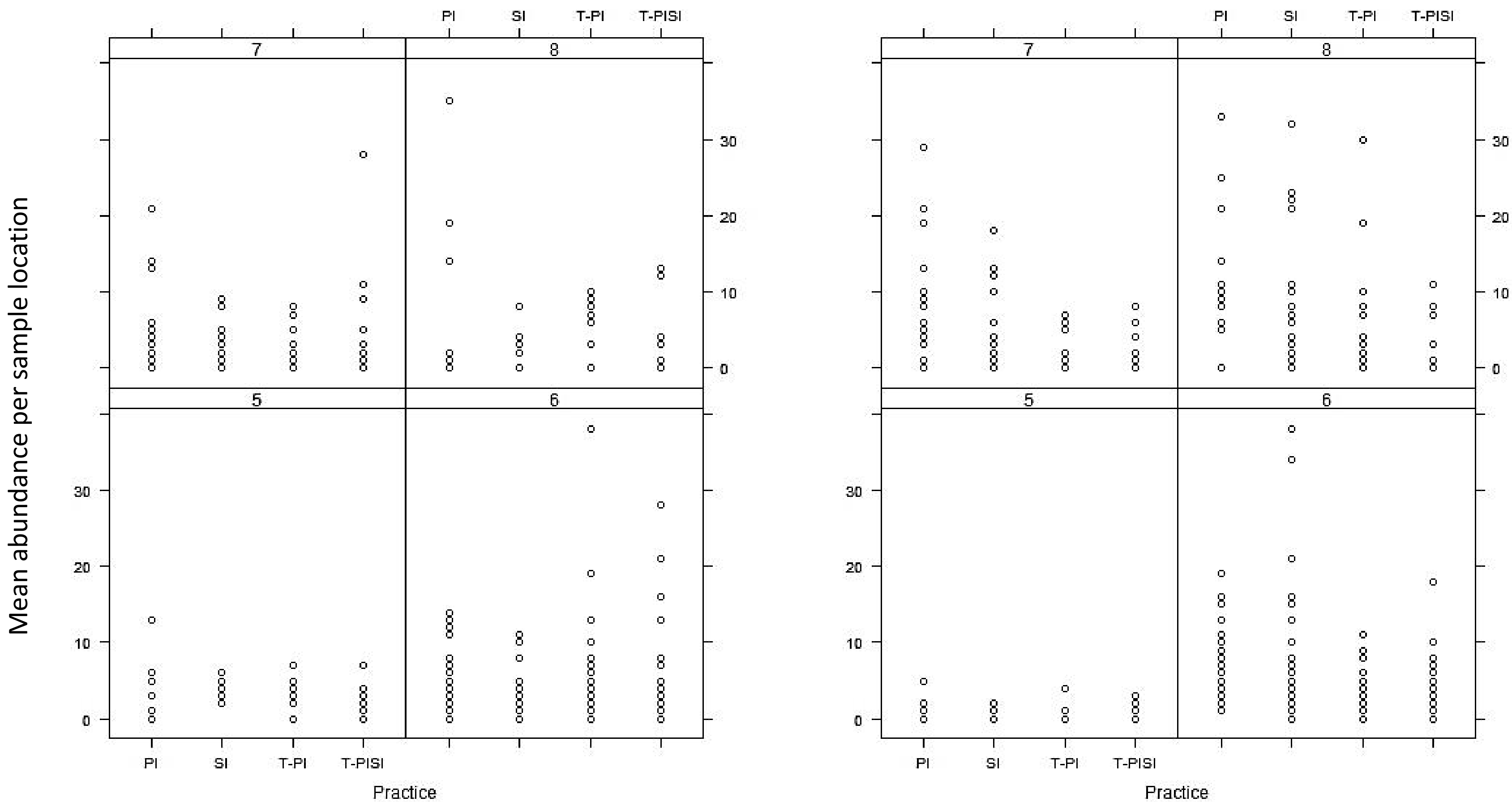
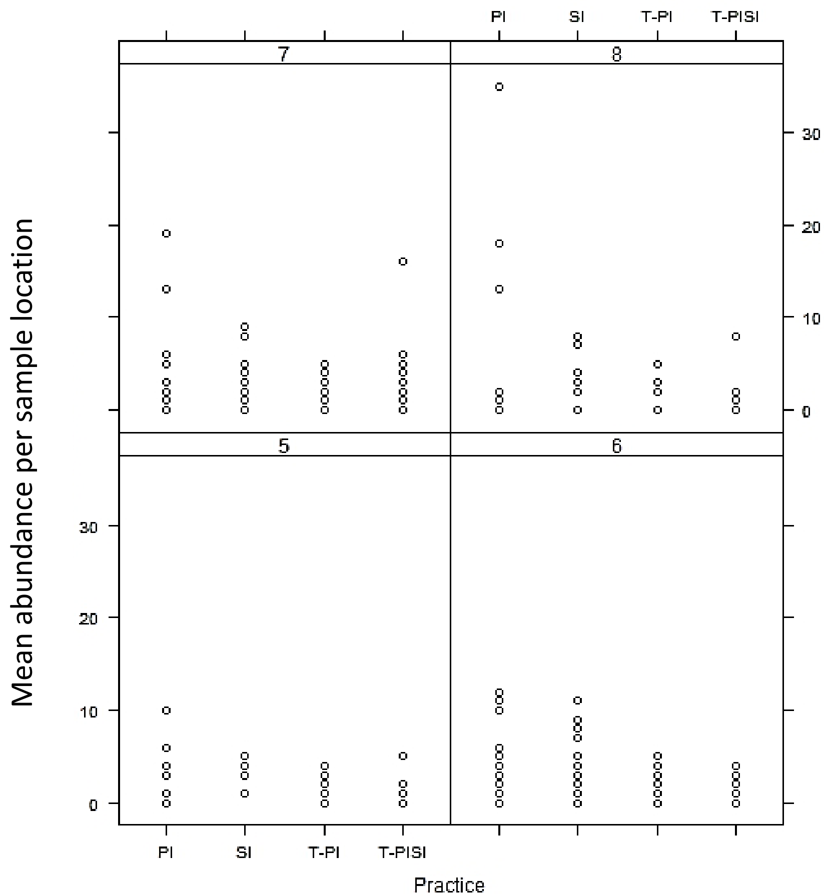



Figure 31. Abundance of all bees captured at each trap set location during May (denoted as 5), June (6), July (7), August (8) in 2013 (Left) and 2014 (Right). Practice: T-PI, 10-year old control stand similar to CP3 planting; T-PISI, 10-year old stand with intercropped switchgrass; PI, 5-year old control stand similar to CP3 planting; SI, 5-year old stand with intercropped switchgrass.



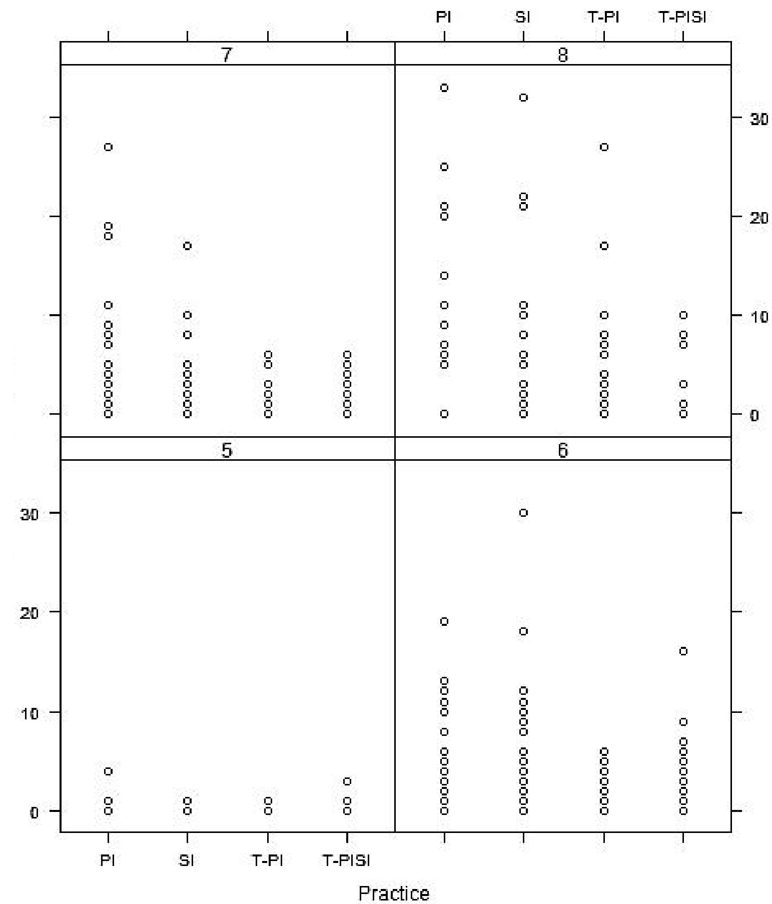




Figure 32. Abundance of ground nesting bees captured at each trap set location during May (denoted as 5), June (6), July (7), August (8) in 2013 (Left) and 2014 (Right). Practice: T-PI, 10-year old control stand similar to CP3 planting; T-PISI, 10-year old stand with intercropped switchgrass; PI, 5-year old control stand similar to CP3 planting; SI, 5-year old stand with intercropped switchgrass.

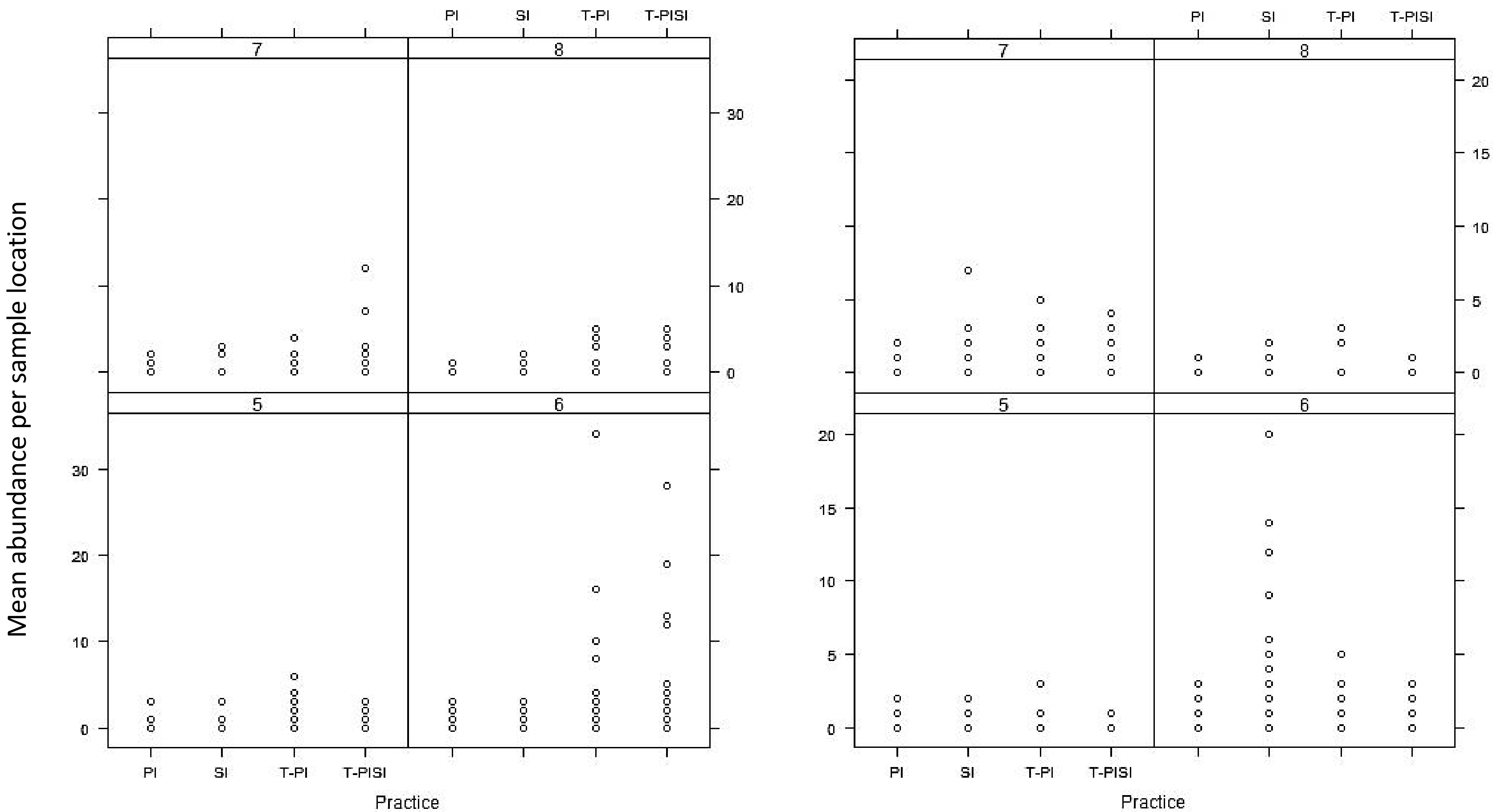



Figure 33. Abundance of non-ground nesting bees captured at each trap set location during May (denoted as 5), June (6), July (7), August (8) in 2013 (Left) and 2014 (Right). Practice: T-PI, 10-year old control stand similar to CP3 planting; T-PISI, 10-year old stand with intercropped switchgrass; PI, 5-year old control stand similar to CP3 planting; SI, 5-year old stand with intercropped switchgrass.

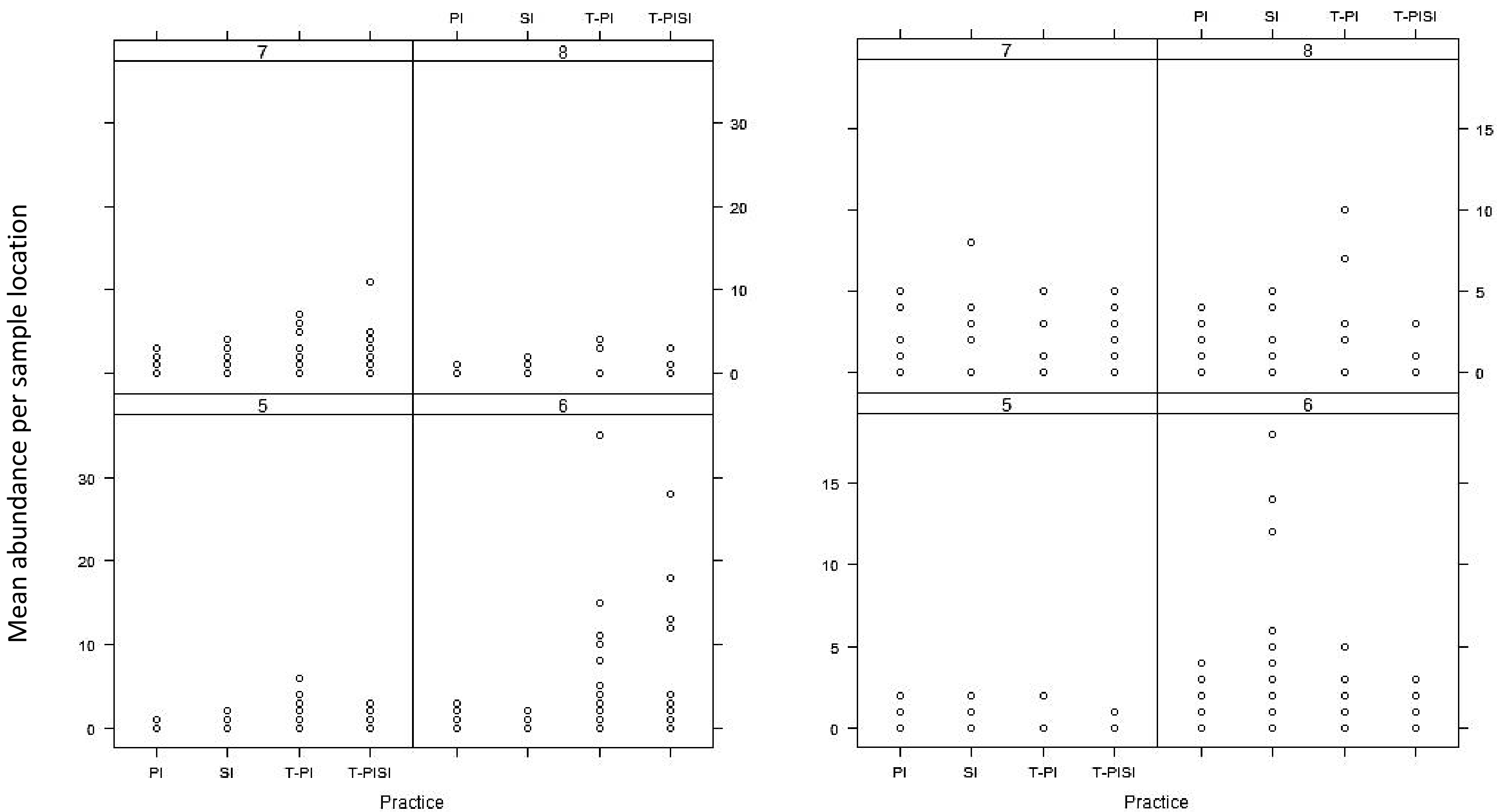



Figure 34. Abundance of Apidae captured at each trap set location during May (denoted as 5), June (6), July (7), August (8) in 2013 (Left) and 2014 (Right). Practice: T-PI, 10-year old control stand similar to CP3 planting; T-PISI, 10-year old stand with intercropped switchgrass; PI, 5-year old control stand similar to CP3 planting; SI, 5-year old stand with intercropped switchgrass.

Table 15. Summary of F-values (P-values in parenthesis) from a general linear mixed model with practice as fixed effect and stand as random effect. Significant practice effects (P<0.1) are bolded.

| Response variable | 2013 | | | | 2014 | | | |
|---|---|---|---|---|---|---|---|---|
| | May[1] | June | July | August | May[1] | June[1,2] | July | August |
| Total | 0.600 | 0.147 | 0.306 | 0.113 | 0.0631 | 2.803 | 1.440 | **7.609** |
| | (0.633) | (0.929) | (0.821) | (0.950) | (0.615) | (0.108) | (0.302) | **(0.010)** |
| Ground | 1.695 | 2.711 | 0.767 | 0.346 | 0.562 | **3.076** | 2.577 | **6.927** |
| | (0.245) | (0.115) | (0.543) | (0.793) | (0.655) | **(0.091)** | (0.127) | **(0.013)** |
| Non-ground | 1.266 | **3.237** | 0.491 | **4.578** | 0.156 | 1.134 | 0.035 | 1.480 |
| | (0.350) | **(0.082)** | (0.698) | **(0.038)** | (0.923) | (0.392) | (0.991) | (0.292) |
| Apidae | **5.650** | **5.430** | 0.731 | **4.900** | 0.903 | 1.154 | 0.113 | 0.559 |
| | **(0.022)** | **(0.025)** | (0.562) | **(0.032)** | (0.481) | (0.385) | (0.950) | (0.657) |

[1] One sampling occasion
[2] Three sampling occasions

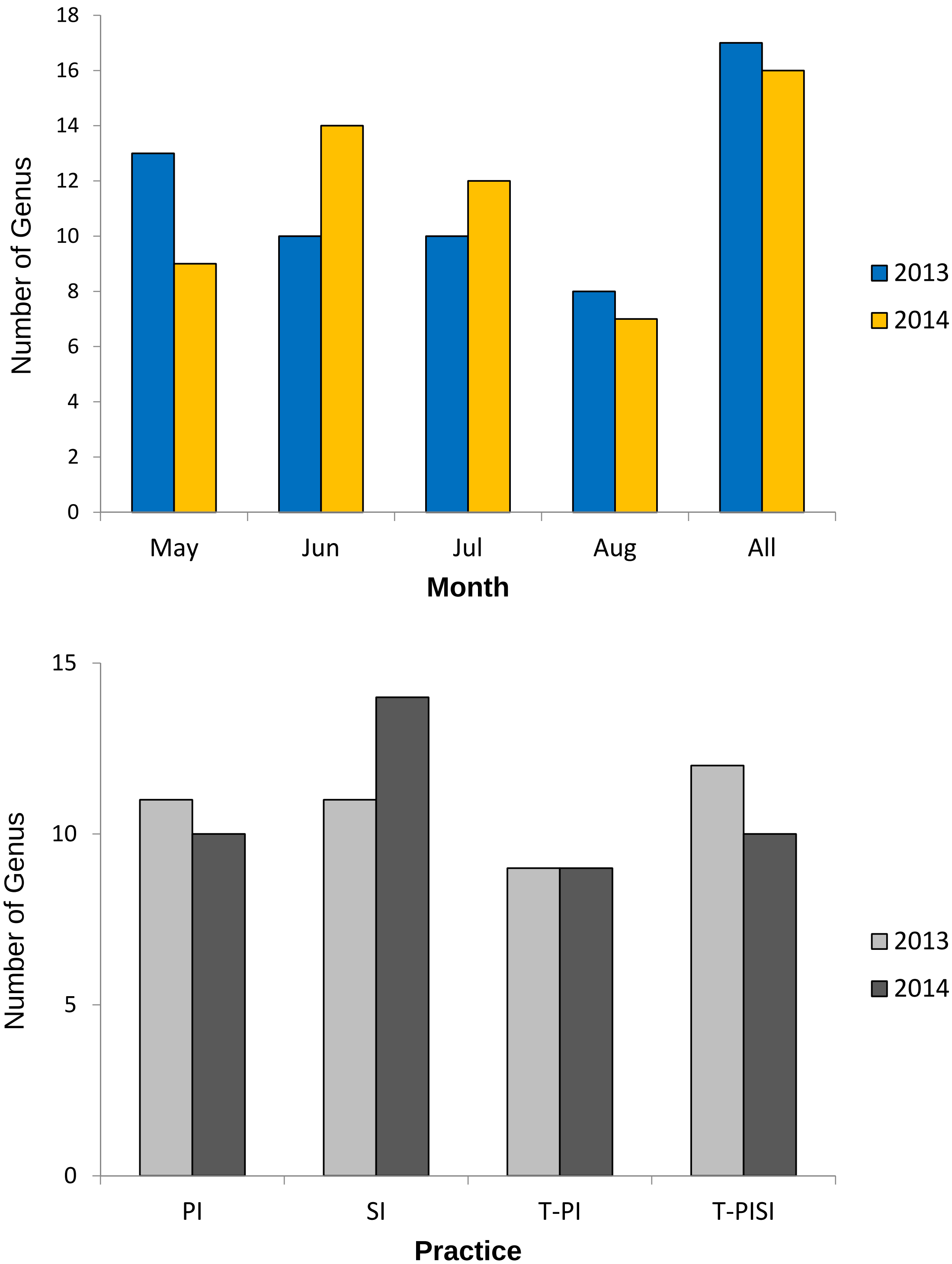



Figure 35. Genus richness (number of genera) of bees by month (upper) and practice (lower). Practice: CONT, 10-year old control stand similar to CP3 planting; PILO, 10-year old stand with intercropped switchgrass; PINE, 5-year old control stand similar to CP3 planting; SWIN, 5-year old stand with intercropped switchgrass.

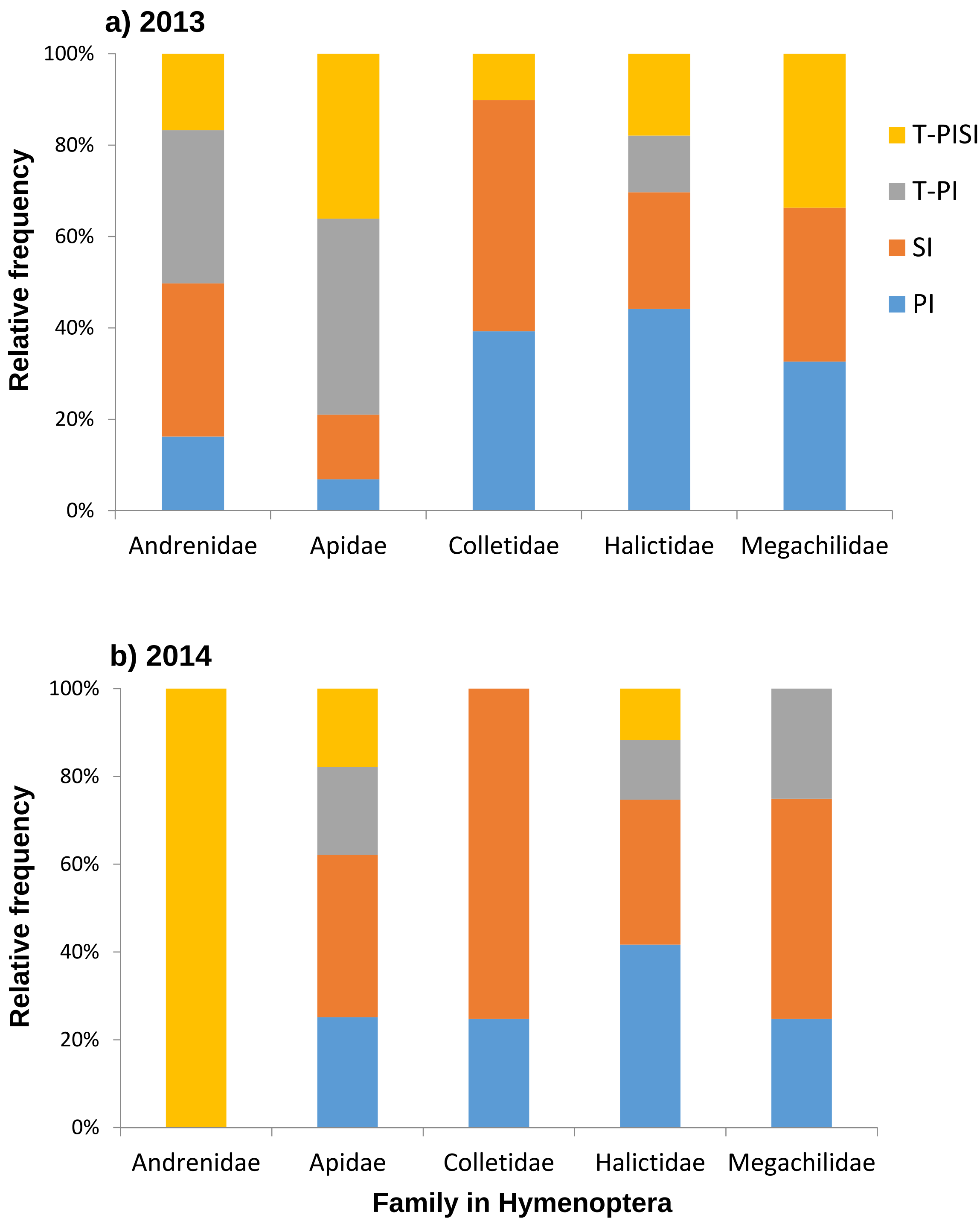
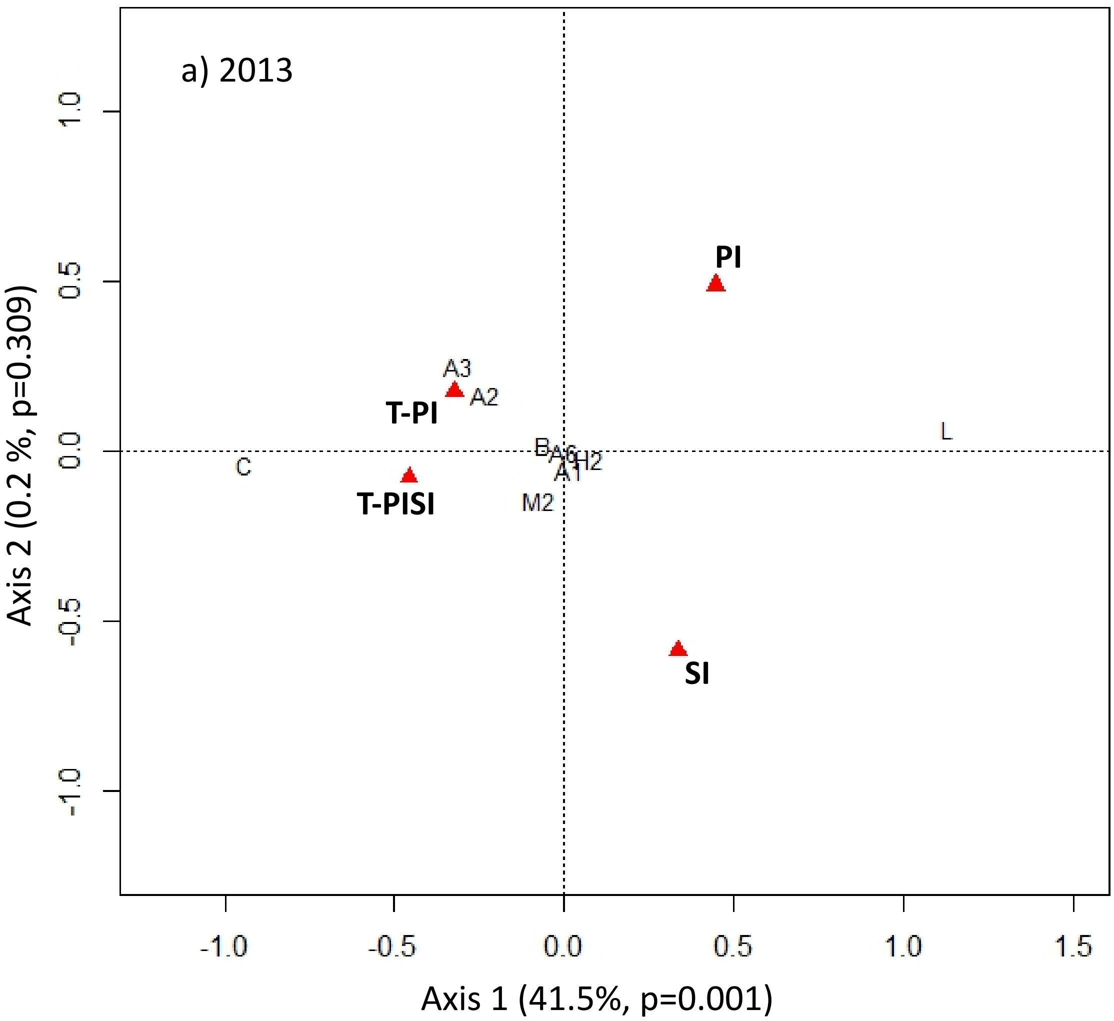



Figure 36. Relative proportion of abundance of five bee families at each practice. Note that only 4 individuals of Andrenidae were captured in 2013 and only 1 individual in 2014. Practice: CONT, 10-year old control stand similar to CP3 planting; PILO, 10-year old stand with intercropped switchgrass; PINE, 5-year old control stand similar to CP3 planting; SWIN, 5-year old stand with intercropped switchgrass.


a) 2013
PI
A3
A2
T-PI
L
B
A6
H2
C
A1
T-PISI
M2
SI
1.0
0.5
0.0
-0.5
-1.0
Axis 2 (0.2 %, p=0.309)
-1.0
-0.5
0.0
0.5
1.0
1.5
Axis 1 (41.5%, p=0.001)

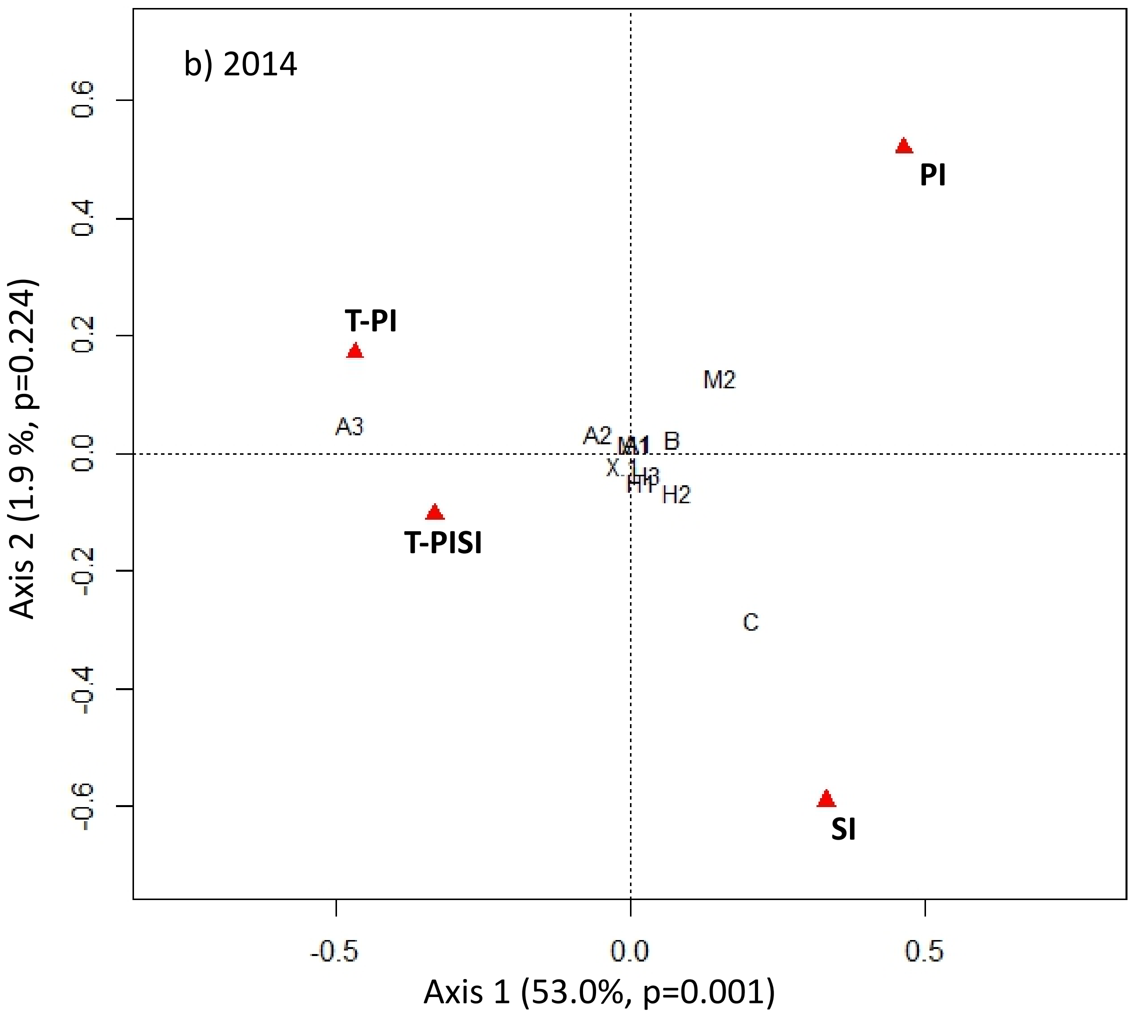



Figure 37. Redundancy analysis (RDA) biplot of four practices and bee genera at Scooba site in 2013 (a) and 2014 (b). Variance (%) explained by each of the first two axis and *p* values calculated from permutation test of 999 iterations are shown in parenthesis. Practice: T-PI, 10-year old control stand similar to CP3 planting; T-PISI, 10-year old stand with intercropped switchgrass; PI, 5-year old control stand similar to CP3 planting; SI, 5-year old stand with intercropped switchgrass. Genus: A1, Apis; A2, Augochlora; A3, Augochlorella; A6, Andrena; B, Bombus; C, Certina; H1, Halictus; H2, Hylaeus; L, Lasioglossum; M1, Megachile; M2, Melissodes; X, Xylocopa.

Table 16. Order, family, and guild classification (based on foraging strategy) of all insects detected at Scooba site, Mississippi during 2013-2014.

| **Order** | **Family** | **Foraging strategy** |
|---|---|---|
| Coleoptera | Buprestidae | Pollinators |
| | Cerambycidae | Herbivores |
| | Meloidae | Predators/parasites/parasitoids |
| | Mordellidae | Others/omnivores |
| Diptera | Bombyliidae | Pollinators, Predators/parasites/parasitoids |
| | Dolichopodidae | Predators/parasites/parasitoids |
| | Syrphidae | Predators/parasites/parasitoids, Pollinators |
| | Tabanidae | Pollinators |
| Hemiptera | Alydidae | Herbivores (seeds) |
| | Coreidae | Herbivores |
| | Reduviidae | Predators/parasites/parasitoids |
| Homoptera | Cercopidae | Herbivores |
| | Cicadellidae | Herbivores |
| | Membracidae | Herbivores |
| Hymenoptera | Andrenidae | Pollinators |
| (Bees) | Apidae | Pollinators |
| | Colletidae | Pollinators |
| | Halictidae | Pollinators, Herbivores |
| | Megachilidae | Pollinators, Predators/parasites/parasitoids |
| Hymenoptera | Chrysididae | Predators/parasites/parasitoids |
| (Non-bees, Wasps) | Crabronidae | Predators/parasites/parasitoids |
| | Pompilidae | Predators/parasites/parasitoids |

| | | |
|---|---|---|
| | Scoliidae | Pollinators |
| | Sphecidae | Predators/parasites/parasitoids |
| | Tiphiidae | Pollinators |
| | Vespidae | Predators/parasites/parasitoids |
| Lepidoptera | Hesperiidae | Pollinators, Herbivores |
| | Sphingidae | Pollinators |
| | Satyridae | Pollinators |

Table 17. Number of individuals of each family of insects detected at Scooba site, Mississippi during 2013-2014.

| **Family** | **2013** | | | | **2014** | | | | **Total** |
|---|---|---|---|---|---|---|---|---|---|
| | May[1] | Jun | Jul | Aug | May[1] | Jun[2] | Jul | Aug | |
| Alydidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Andrenidae | 5 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 7 |
| Apidae | 37 | 226 | 115 | 33 | 16 | 186 | 113 | 67 | 793 |
| Bombyliidae | 7 | 0 | 0 | 0 | 11 | 0 | 0 | 1 | 19 |
| Buprestidae | 12 | 24 | 9 | 0 | 19 | 83 | 8 | 1 | 156 |
| Cerambycidae | 9 | 3 | 0 | 0 | 1 | 6 | 0 | 0 | 19 |
| Cercopidae | 2 | 1 | 0 | 2 | 0 | 0 | 0 | 1 | 6 |
| Chrysididae | 0 | 0 | 4 | 2 | 1 | 4 | 4 | 2 | 17 |
| Cicadellidae | 24 | 1001 | 730 | 318 | 28 | 605 | 374 | 605 | 3685 |
| Colletidae | 1 | 8 | 1 | 0 | 0 | 5 | 1 | 6 | 22 |
| Coreidae | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 2 |
| Crabronidae | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 3 |
| Dolichopodidae | 1462 | 805 | 449 | 488 | 659 | 753 | 643 | 359 | 5618 |
| Halictidae | 79 | 198 | 131 | 164 | 19 | 428 | 210 | 445 | 1674 |
| Hesperiidae | 6 | 36 | 75 | 11 | 3 | 16 | 11 | 19 | 177 |
| Megachilidae | 2 | 0 | 0 | 1 | 0 | 8 | 4 | 0 | 15 |
| Meloidae | 2 | 10 | 12 | 6 | 1 | 2 | 1 | 2 | 36 |
| Membracidae | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 7 | 9 |
| Mordellidae | 314 | 788 | 151 | 38 | 62 | 323 | 80 | 22 | 1778 |
| Pompilidae | 0 | 0 | 23 | 5 | 1 | 2 | 3 | 4 | 38 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Reduviidae | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 4 |
| Satyridae | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 2 |
| Scoliidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Sphecidae | 4 | 6 | 3 | 1 | 2 | 15 | 5 | 5 | 41 |
| Sphingidae | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Syrphidae | 12 | 43 | 219 | 430 | 2 | 209 | 208 | 235 | 1358 |
| Tabanidae | 7 | 22 | 1 | 2 | 3 | 13 | 4 | 3 | 55 |
| Tiphiidae | 0 | 0 | 1 | 1 | 0 | 6 | 0 | 0 | 8 |
| Vespidae | 4 | 5 | 6 | 2 | 0 | 5 | 1 | 2 | 25 |
| **Total** | 1990 | 3177 | 1933 | 1504 | 831 | 2673 | 1674 | 1789 | 15571 |

1 One sampling occasion
2 Three sampling occasions

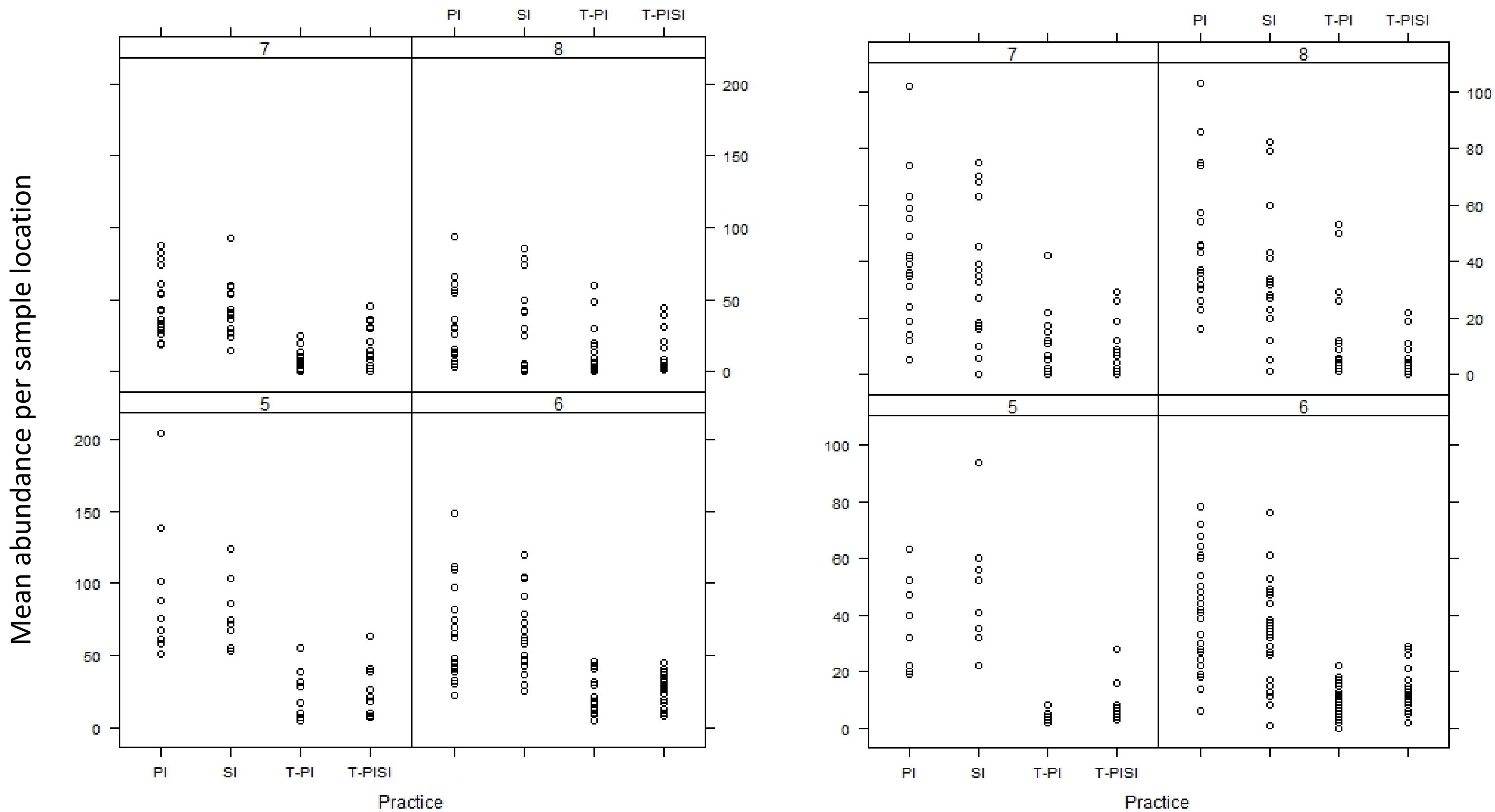



Figure 38. Abundance of all insects captured at each trap set location during May (denoted as 5), June (6), July (7), August (8) in 2013 (Left) and 2014 (Right). Practice: T-PI, 10-year old control stand similar to CP3 planting; T-PISI, 10-year old stand with intercropped switchgrass; PI, 5-year old control stand similar to CP3 planting; SI, 5-year old stand with intercropped switchgrass.

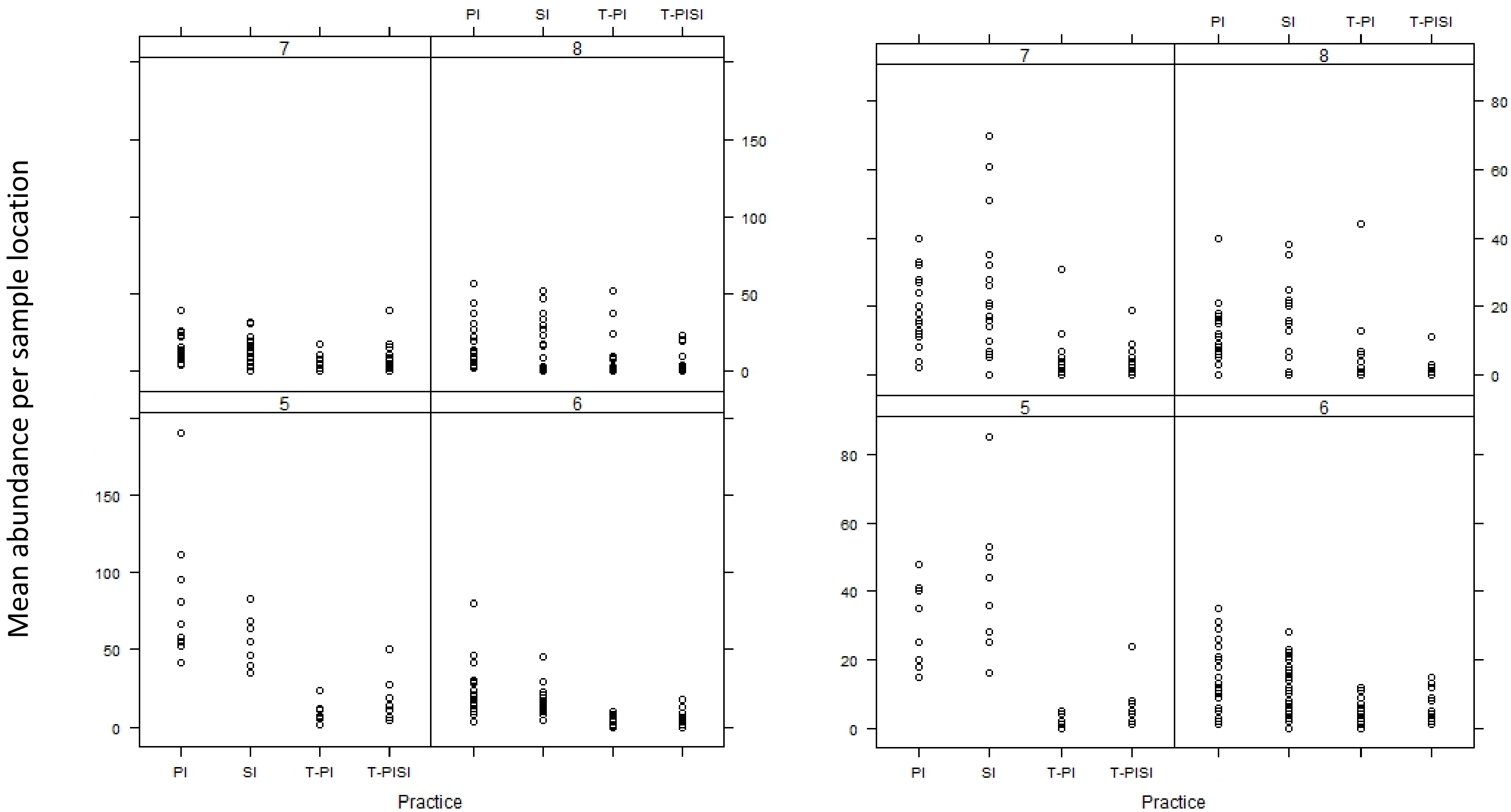



Figure 39. Abundance of predators/parasites/parasitoids captured at each trap set location during May (denoted as 5), June (6), July (7), August (8) in 2013 (Left) and 2014 (Right). Practice: T-PI, 10-year old control stand similar to CP3 planting; T-PISI, 10-year old stand with intercropped switchgrass; PI, 5-year old control stand similar to CP3 planting; SI, 5-year old stand with intercropped switchgrass.

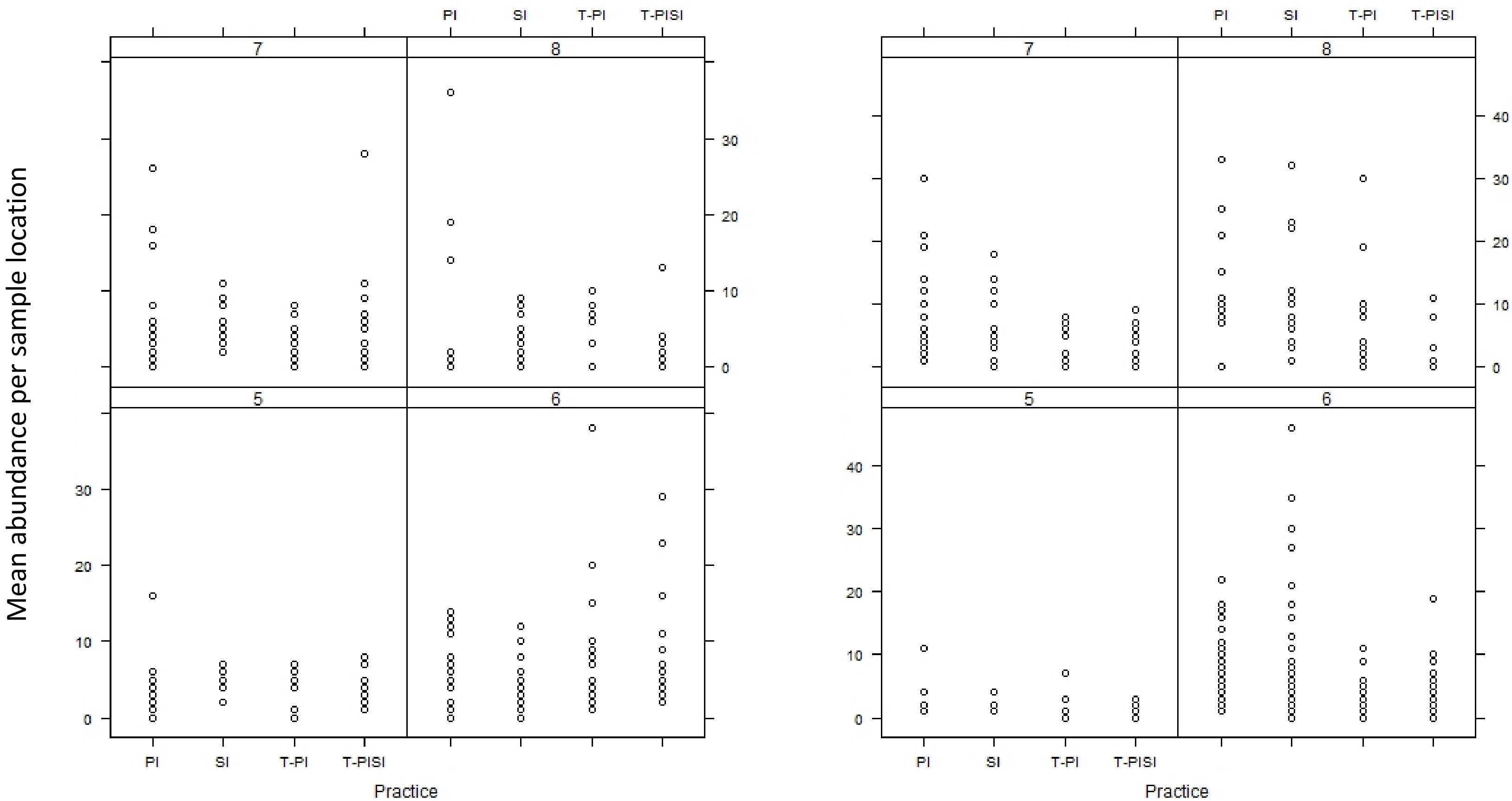



Figure 40. Abundance of pollinators captured at each trap set location during May (denoted as 5), June (6), July (7), August (8) in 2013 (Left) and 2014 (Right). Practice: T-PI, 10-year old control stand similar to CP3 planting; T-PISI, 10-year old stand with intercropped switchgrass; PI, 5-year old control stand similar to CP3 planting; SI, 5-year old stand with intercropped switchgrass.

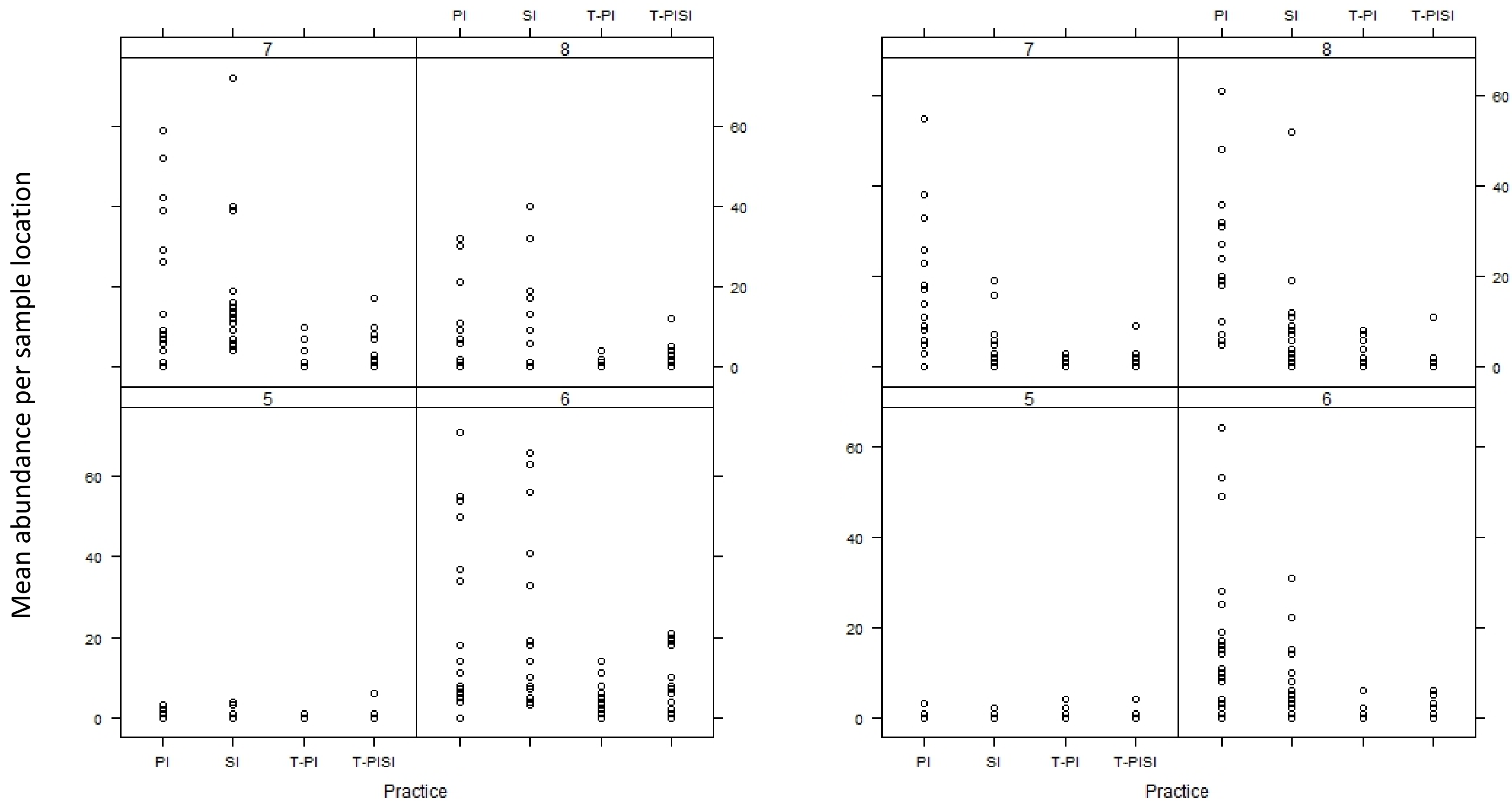
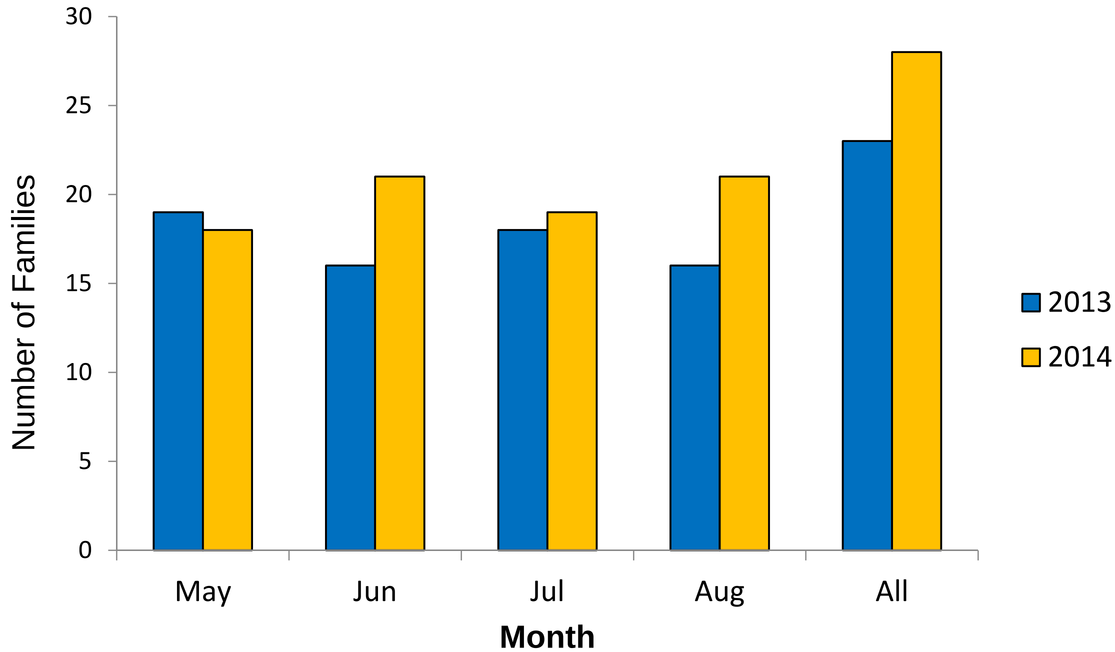



Figure 41. Abundance of herbivores captured at each trap set location during May (denoted as 5), June (6), July (7), August (8) in 2013 (Left) and 2014 (Right). Practice: T-PI, 10-year old control stand similar to CP3 planting; T-PISI, 10-year old stand with intercropped switchgrass; PI, 5-year old control stand similar to CP3 planting; SI, 5-year old stand with intercropped switchgrass.



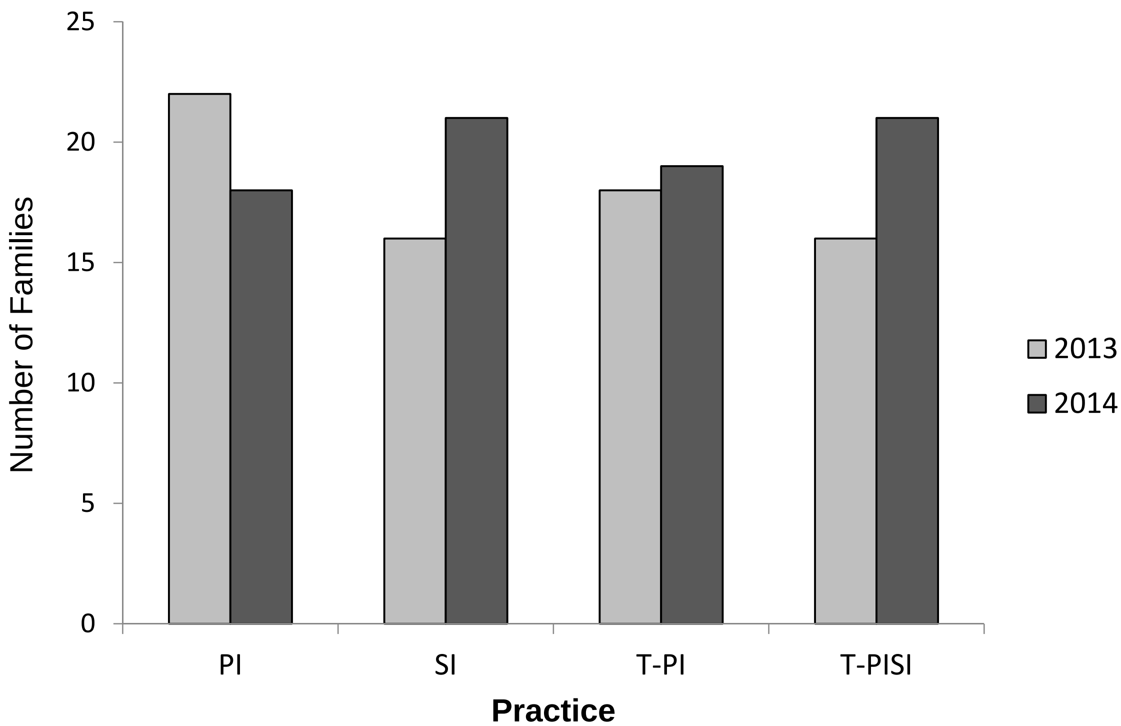




Figure 42. Family richness (number of families) of insects by month (upper) and practice (lower). Practice: T-PI, 10-year old control stand similar to CP3 planting; T-PISI, 10-year old stand with intercropped switchgrass; PI, 5-year old control stand similar to CP3 planting; SI, 5-year old stand with intercropped switchgrass.

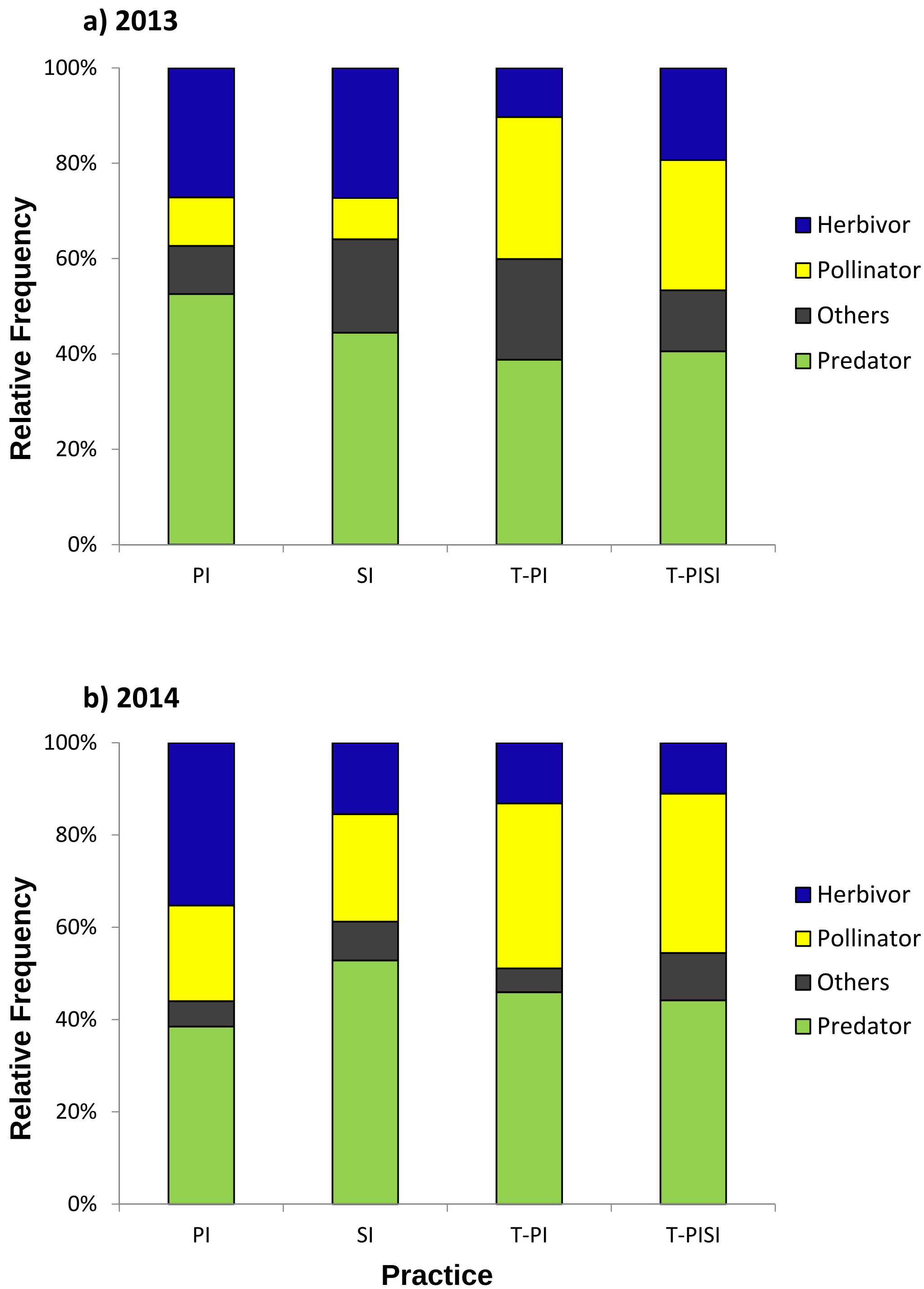



Figure 43. Relative proportion of abundance of insect foraging guilds at each practice. "Predator" represents predators/parasites/parasitoids foraging guild. Practice: T-PI, 10-year old control stand similar to CP3 planting; T-PISI, 10-year old stand with intercropped switchgrass; PI, 5-year old control stand similar to CP3 planting; SI, 5-year old stand with intercropped switchgrass.

4. Bloom-time Survey

Among 11 major blooming plant species, 9 species (6 species at each of two sites, Prairie site and Bryan Farms site) were detected during 2012 -2013 (Table 18). Roundheaded lespedeza (*Lespedeza capitata*) and Showy ticktrefoil (*Desmodium canadense*) were not observed at any of those study sites. Total number of detections of these 9 plant species was 377, however most of them were observed at Bryan Farms site: Prairie sites, 40 detections; Brayan Farms site, 255 and 82 detections in 2012 and 2013, respectively. Low total number of detections at Bryan site during 2013 could be affected by low sampling occasions because only 10 visits were made in 2013 whereas there were two times more visits (19 visits) in 2012. While butterfly milkweed and Black-eyed Susan were more often present at Prairie site, Tick-seed sunflower and Illinois bundleflower were detected mostly at Bryan Farms site.

During survey periods, 31 other blooming species (excluding unknown species) were also observed (Table 19). Total number of detections of those plants was 3.6 times higher than the detections of major blooming species. Early buttercup, Carolina horsenettle, and Brazilian vervain were frequently detected, comprising 50% of total detections of other blooming species.

Each of 9 major plant species showed variations in bloom time (Fig. 44). While Tick-seed sunflower and Partridge pea bloomed throughout summer and fall, flowering Purple prairie clover and Purple coneflower were observed only in May. Illinois bundleflowers also bloomed for a longer period, from May to September. Blooming period of other three species was relatively short (2 months).

Seasonal variations were also found in richness of major blooming species (Fig. 45). At Prairie site, mean richness was high in June and declined throughout summer. At Bryan Farms site, mean richness was greater in June and August both years. While mean richness decreased between September and other in 2012 (no blooming plants in October), it did not change in 2013. In addition, total number of detections varied with practices (Fig. 46). At Prairie site, ungrazed native warm season pasture showed relatively higher detections of major blooming plants than other practices. At Bryan Farms site, significantly higher detections were observed at NWSG mix (both single biomass harvest and multiple biomass harvest, particularly single biomass harvest).

Table 18. Total number of detections (presences) of major blooming species observed across all practices at Prairie site (2012) and at Bryan farm site (2012-2013).

| Scientific Name | Common Name | Prairie | Bryan Farm | | Total |
|---|---|---|---|---|---|
| | | 2012 | 2012 | 2013 | |
| *Asclepias tuberosa* | Butterfly milkweed | 16 | 0 | 0 | 16 |
| *Bidens polylepis* | Tick-seed sunflower | 0 | 145 | 34 | 179 |
| *Chamaecrista fasciculate* | Partridge pea | 3 | 20 | 21 | 44 |
| *Dalea purpurea* | Purple prairie clover | 1 | 0 | 0 | 1 |
| *Desmanthus illinoensis* | Illinois bundleflower | 5 | 82 | 24 | 111 |
| *Echinacea purpurea* | Purple coneflower | 1 | 0 | 0 | 1 |
| *Helianthus maximiliani* | Maximilian sunflower | 0 | 6 | 0 | 6 |
| *Ratibida pinnata* | Grey-headed coneflower | 0 | 0 | 3 | 3 |
| *Rudbeckia hirta* | Black-eyed Susan | 14 | 2 | 0 | 16 |
| | Total | 40 | 255 | 82 | 377 |

Table 19. Total number of detections (presences) of major blooming species observed across all practices at Prairie site (2012) and at Bryan farm site (2012-2013).

| Scientific Name | Common Name | Prairie | Bryan Farms | | Total |
|---|---|---|---|---|---|
| | | 2012 | 2012 | 2013 | |
| *Agalinis purpurea* | Purple gerardia | 2 | 17 | 0 | 19 |
| *Ambrosia artemisiifolia* | Annual ragweed | 5 | 14 | 25 | 44 |
| *Ambrosia trifida* | Great ragweed | 3 | 0 | 10 | 13 |
| *Aster patens* | Late purple aster | 2 | 22 | 4 | 28 |
| *Aster Pilosis* | Frost aster | 51 | 18 | 82 | 151 |
| *Taraxacum* spp. | Dandelion | 0 | 0 | 2 | 2 |
| *Diodia* spp. | Buttonweed | 0 | 12 | 0 | 12 |
| *Diodia teres* | Poorjoe | 0 | 2 | 0 | 2 |
| *Erigeron annuus* | Daisy fleabane | 0 | 7 | 0 | 7 |
| *Eupatorium capillifolium* | Dogfennel | 3 | 0 | 4 | 7 |
| *Eupatorium* spp. | | 0 | 37 | 8 | 45 |
| *Eupatorium Serotinum* | Late boneset or late thoroughwort | 23 | 0 | 18 | 41 |
| *Helenium amarum* | Yellowdicks | 0 | 0 | 2 | 2 |
| *Conyza canadensis* | Horsetail | 24 | 17 | 12 | 53 |
| *Iva annua* | Annual marsh elder | 2 | 23 | 13 | 38 |
| *Jacquemontia tamnifolia* | Hairy clustervine | 0 | 2 | 0 | 2 |
| *Ludwigia* spp. | Seed box | 0 | 17 | 0 | 17 |
| *Ipomoea pandurata* | Morning glory | 0 | 17 | 0 | 17 |
| *Pluchea* | Stinkweed | 1 | 31 | 0 | 32 |

| | | | | | |
|---|---|---|---|---|---|
| *Polygonium Hydropiperoids* | Swamp smartweed | 0 | 0 | 1 | 1 |
| *Ranunculus fascicularis* | Early buttercup | 108 | 25 | 56 | 189 |
| *Rubus trivialis* | Southern dewberry | 8 | 0 | 0 | 8 |
| *Polygonum* spp. | Smartweed | 0 | 31 | 0 | 31 |
| *Sesbania* spp. | Sicklepod | 0 | 22 | 0 | 22 |
| *Solanum carolinense* | Carolina horsenettle | 83 | 28 | 67 | 178 |
| *Solidago canadensis* | Canada goldenrod | 22 | 28 | 10 | 60 |
| *Trifolium repens* | White clover | 0 | 0 | 3 | 3 |
| *Verbena brasiliensis* | Brazilian vervain | 66 | 58 | 193 | 317 |
| *Vernonia gigantea* | Giant ironweed | 0 | 0 | 3 | 3 |
| | Wild Onion | 13 | 0 | 0 | 13 |
| Unknown Thistle | Thistle | 0 | 0 | 2 | 2 |
| Unknown species | | 3 | 4 | 3 | 10 |

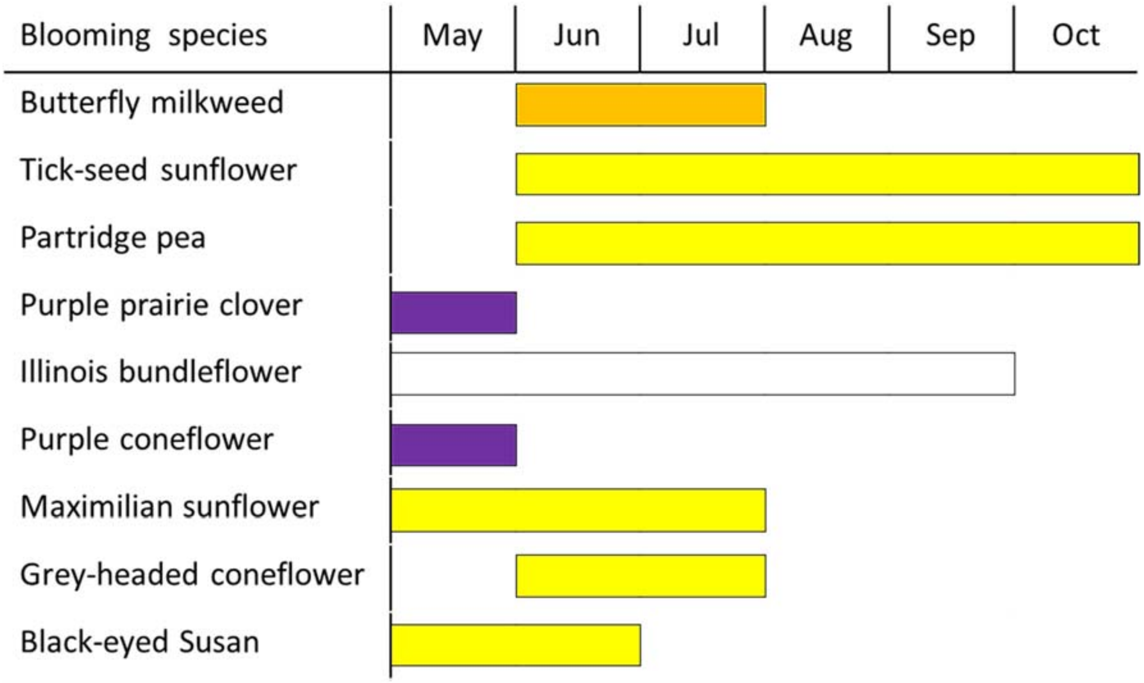



Figure 44. Blooming period of nine major plant species found across all study sites (Prairie site and Bryan farm site) during 2012-2013.

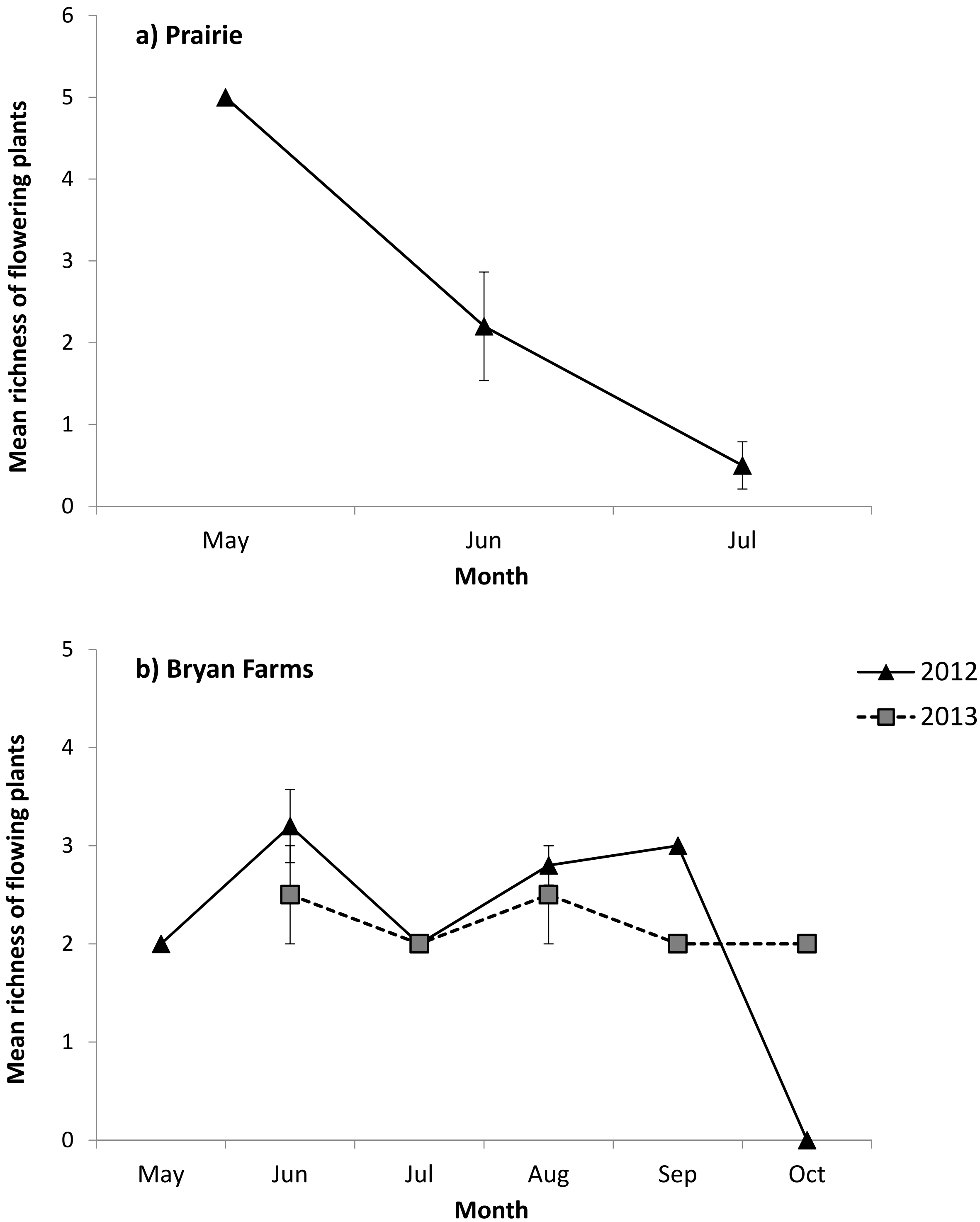



Figure 45. Seasonal variations in mean richness of major blooming plants. Bars represent standard errors (SE). Two to five surveys were conducted each month except May (only one survey). No blooming major species were found at Prairie site (a) during August-October.

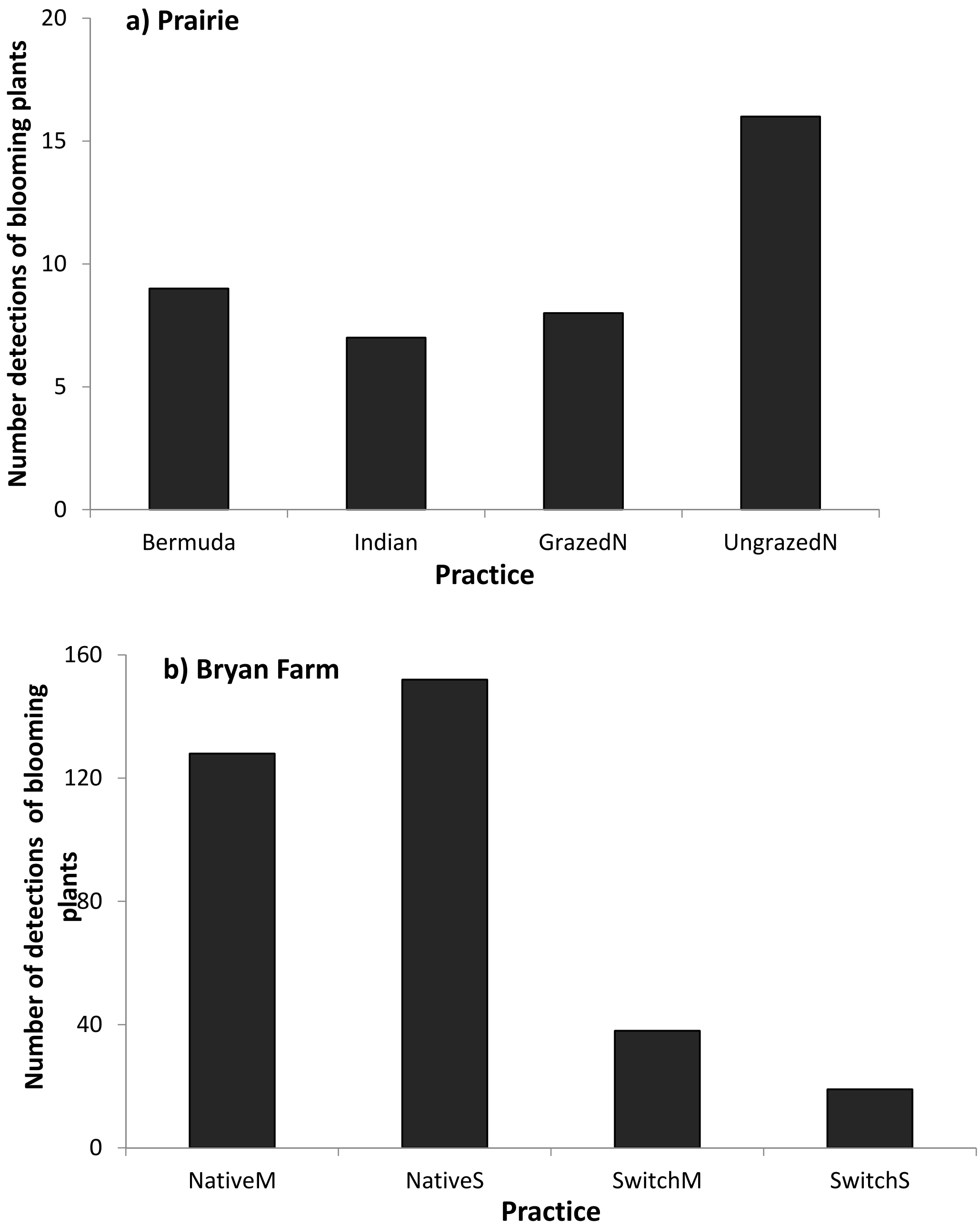



Figure 46. Total number of detections of major blooming plants observed across all pastures or fields at each practice for two years (2012-2013; only 2012 at Prairie site). Practice: Bermuda, Grazed Bermudagrass pasture (Practice code # 528); GrazedN, Grazed native warm season pasture (Practice code # 528 & 550); Indian, Grazed Indiangrass, (Practice code # 528 & 550); UngrazedN, Ungrazed native warm season pasture (Practice code # 550); NativeS,native warm season grass (NWSG) mix with a single biomass harvest; NativeM, NWSG mix with multiple biomass harvests (Practice code # 511); SwitchS, Switchgrass monoculture with a single biomass harvest; SwitchM, Switchgrass monoculture with multiple biomass harvests (Practice code # 511).

Conclusions and recommendations

This project investigated how biofuel crop and harvest, grazing, and planting mixture (native warm season grasses, Indiangrass and Bermudagrass) influenced insect community with an emphasis on pollinators (bees). It also included a wide range of conservation practices or treatments. Given most research has been focused on a few aspects of conservation practices, this project provided comprehensive information about effects of conservation practices on insect community in agricultural landscapes.

1. Grazing vs. ungrazing and native grass vs. non-native grass

The analysis results at Prairie site showed that ungrazed mixture of native warm season grasses (NWSG planting) and Indaingrass monoculture were more suitable in improving abundance of pollinators than other practices, especially during summer when pollinators were abundant. Considering bee genera associated with ungrazed NWSG planting were all non-ground nesting bees, ungrazing practice could be important to create nesting habitat for those bees. Insect community at ungrazed NWSG planting was also composed of more pollinators, indicating relative importance of ungrazed NWSG planting in provision of pollination service. Higher abundance of pollinators at Indiangrass monoculture suggested that native grass monoculture could be beneficial for pollinators compared to non-native grass (Bermudagrass) monoculture. Abundance of pollinators at Indiangrass monoculture was also higher than at grazed NWSG planting and there was insignificant difference between Bermudagrass monoculture and grazed NWSG planting. This indicated that unlike the pattern expected in row crop monoculture systems (Cane and Tepedino, 2001), monoculture in pasture production systems may not negatively influence on pollinators.

2. Multiple harvests vs. single harvest and NWSG planting vs. switchgrass monoculture

The results from Bryan Farms site suggested relatively stronger effect of biofuel crop type (NWSG planting vs. switchgrass monoculture) on pollinators than harvest frequency (multiple vs. single). While abundance of total bees and two nesting guilds was not significantly influenced by harvest frequency (based on the results of 2012), it showed a significant response to crop type. In 2011 which no harvest was applied to, abundance of bees tended to be high at switchgrass monoculture during May-June and at NWSG planting during July-November (except October). This seems to suggest that switchgrass monoculture may be a more effective practice for management of pollinators when insect abundance peaks. However, higher abundance at NWSG planting during late summer-fall indicates that NWSG planting may support pollinators for a longer period. In addition, greater diversity of insect families at NWSG planting may indicate that NWSG planting could be more appropriate as biodiversity conservation management for insects compared to switchgrass monoculture.

It should be noted that predator/parasite/parasitoid foraging insects were the most dominant in all practices at Bryan Farms site (and Prairie site), whereas abundance of herbivores was low. One of the concerns related to establishment of biofuel crops and other agricultural crops is potential damages from arthropod pests (e.g., herbivore insects) (Landis and Werling, 2010). Predator/parasite/parasitoid insects play an important role in pest control. Dominance of the foraging guild suggests that yield losses by pest problems would be minimal in biofuel production systems tested in this project.

3. Effect of stand age and switchgrass intercropping

The findings at Scooba site indicated that pine stand age was an important factor influencing abundance of pollinators and all insects: mostly higher abundance of pollinators at 5-year-old pine stands (relatively open canopy) than at 10-year-old pine stands (closed canopy). In pine forests, age of pine stand is one of major considerations for wildlife management due to strong correlation between stand age and amount of canopy cover or closure that determines vegetation structure within a stand (Melchiors, 1991). Thus, significant effect of stand age at Scooba site was expected and consistent with the pattern (low biodiversity and abundance of, e.g., birds) frequently observed at a 10-15-year-old pine stand which forms closed canopy. Unlike the strong effect of stand age, switchgrass intercropping practice did not have either negative or positive effects on pollinators although abundance of pollinators was slightly higher at pine stands without intercropped switchgrass (except June, 2014). However, all insect abundance tended to be higher at 5-year old stand with no switchgrass intercropping both years and at 10-year old stand with switchgrass intercropping during May-June. This suggested that effect of switchgrass intercropping on insect abundance may vary with age of stand. Further study is needed to test this pattern.

4. Flowing plants and their bloom-time

Identification of blooming plants in pastures or row crop fields and description of their bloom time provided basic but crucial information in understanding distribution patterns of pollinators. However, they are rarely studied in Mississippi. Little is known when and what plants bloom in agricultural landscapes in Mississippi. The bloom-time survey results from this project offer baseline information that can be used for the management of pollinators. For example, establishing plant species (Illinois bundleflower, Tick-seed sunflower and Partridge pea) blooming for a long period or a combination (Butterfly milkweed and Black-eyed Susan) of species blooming different time of a year may support pollinators for an extended period time and increase pollination services. However, one should note that the bloom-time of each plant is limited by conservation practices examined in this project. Actual blooming period of some plant species could be longer than the blooming period reported in this project. In addition, more importantly, the bloom-time survey results were not based on observations of pollinator foraging on those plant species. Our results do not convey information about a direct relationship between pollinators and those plant species. Future study that documents the relationship and identifies flowing plants favored by pollinators will be critical to conserve pollinators and increase economic benefits associated with pollination services.

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Appendix A. Insect survey details.

<u>Insect Surveys Conducted with Colored bowl-traps</u>

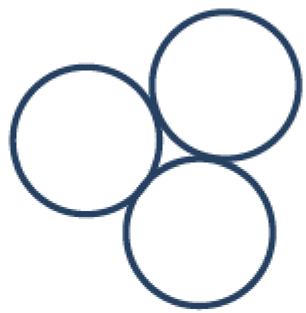
**Supplies List**

- You will need one of each of the following for each trap setup
  - 12 oz school bus yellow bowls
  - 12 oz navy blue bowls
  - 12 oz white bowls
    - Ordered from www.mypapershop.com
  - 10” shelving bracket
    - Can be found in the closet organization section of any hardware store
    - Lowe’s item # 126692
  - 6’ Single Track Standard
    - Can be found in the closet organization section of any hardware store
    - Lowe’s item # 126737 or 107837
- 9 gauge wire
  - Found at any hardware store
- 18 oz Whirl-Pak® write-on bags
  - Ordered online at http://www.enasco.com/product/B01065WA
- 6” PVC cleanout adapter
  - Purchased at any hardware store
- Studded t-posts, 6.5’, 1.25 lbs per foot
  - Purchased at Tractor Supply Company
  - Item # 3609120
- Cattle panel (16’ x 50”)
  - Purchased at Tractor Supply Company
  - Item # 3602077
- Small kitchen strainers or fine mesh dip nets
  - Purchased at any retailer like Wal-Mart or Target
- Laboratory wash bottle
  - Ordered online at www.fishersci.com
- 1 gallon Nalgene jug
  - Ordered online at www.fishersci.com
- Ethanol
  - Purchased at Chemical lab at MSU
- Antibacterial liquid soap
  - Purchased at Wal-Mart
  - Do not use soap with bleach. It will cause discoloration of insects.
- Herbicide backpacks (3 – 4 gallons) or one gallon milk jugs
  - Ordered online at www.amazon.com

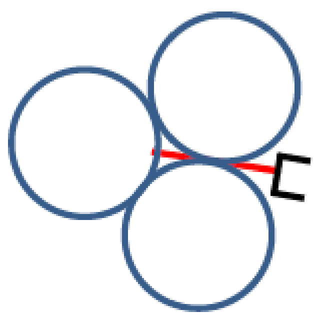
- Water carriers/dispensers that hold 5 gallons or more
    - Ordered online at www.amazon.com

<u>**Colored bowl-trap Protocol**</u>

- **Creation of Trap Setup**
    - Using PVC pipe as a template create three rings of wire, 9 gauge, for each trap.
    - Use j-clips to clamp the ends of the wire ring together. Ensure j-clips are secured to avoid slippage after bowls are in place.
    - Tack weld three rings together such that every ring is in direct contact with each other ring (see below).



- Then take shelving bracket (red below) and cut it to the diameter of a single bowl approximately 6.5”.
- After cutting, weld shelving bracket to the three rings as illustrated below.
- After welding attach bracket and ring contraption to the stand at the desired height. DO NOT weld the ring contraption to the stand because it must remain adjustable.



- **Selecting Trap Locations**
    - Three trap locations per plot will be selected using ArcMap prior to implementing them in the field.
    - It is important to record the GPS coordinates in multiple places (e.g. a database of some sort and GPS unit).
    - If possible place them within 5 m of some sort of vegetation transect.
    - Make sure they are at least 25 m from the edge of the plots to avoid edge effects.
    - Traps should be at least 50 m from each other so they are not visible from each other after the vegetation has grown.

- **Setting up the Traps**
    - Trapping season will run from May 1st through October 31st. It is important to get the traps set up and collected first thing in the morning; this will allow for almost three full days of trapping.
    - Set up bowls in traps twice monthly at least 10 to 14 days apart (i.e. every other week).
    - Do not set up traps if there are thunderstorms in the forecast. Large thunderstorms have the potential to disturb the traps (i.e. cause the bowls to overflow or dislodge a bowl (if severe enough)).
    - Hammer track standard (black above) into position selected before. This will not need to be repeated unless traps have to be removed for some reason (e.g. harvest).
    - Adjust the height of the three rings to be level with either the height of the flowers or vegetation if forbs have not yet begun to flower. Until maximum height of the track standard is reached or the flowers/vegetation stop growing in height.
    - Place one blue, one white, and one yellow bowl in rings of each trap so that each trap has one bowl with each color. Spatial arrangement of colored bowls in each trap is not important.
    - Fill each of the bowls to the brim with a soap-water solution approximately one fluid ounce of soap for every eight gallons of water – using herbicide backpacks or gallon milk jugs to carry soap-water solution in the field (you may need to refill them from the water carriers/dispensers).
    - Repeat until all the traps on the site are set up the same way on the same day.

- **Trap Collection**
    - Three days following trap set up; collect contents of each bowl (e.g. set up the traps on Monday and collect them on Thursday). If traps are left out for more than three days, then soap-water solution will evaporate completely.
    - Before collection, type up uniquely numbered labels such that each label includes site, plot-trap code, date of collection, and GPS coordinates. Labels can be either

printed from a LaserJet printer or hand written with pencil so the writing will not run in the ethanol solution.

- Add labels to whirl-paks prior to collection.
- Using a black sharpie label the outside of the whirl-pak with the plot-trap code. Make sure appropriate trap contents are placed in appropriately labeled whirl-paks.
- Pour the contents of the yellow, blue, and white bowls into a small kitchen strainer or fine mesh dip net.
- Once the contents are out of the soap-water solution place the strainer on the edge of the whirl-pak or invert the dip net into the whirl-pak and wash the contents out with a 70% ethanol mixture, using a laboratory wash bottle.
- Expel excess air from whirl-pak and seal tightly.
- After transferring bowl contents to bags collect bowl (i.e. do not leave them in the traps).
- Repeat until all the traps have been collected.

- **Cages at Prairie, MS**
  - 75% of the plots at the Prairie Research Center are grazed by cattle thus we have to exclude cattle from the trap setups.
  - Using a post drive hammer three t-posts in a triangle around the trap setup.
  - Wrap a single cattle panel around the three t-posts and secure cattle panels to t-posts with wire.

Appendix B. Bloom-time survey protocol.

# Bloom-time Survey Protocol

Each summer a bloom-time survey is to be conducted to provide a blooming time-line of flowering plants that are valuable to important pollinator species in agricultural conservation fields. This survey will consist of weekly transects through planted conservation fields to assess seasonal variation in flower bloom. A list of target flowering species is provided below that was derived from forb species intentionally planted in Native Warm Season Grass plots and CP33 buffers combined with species typically not planted but frequently present and of value to pollinators.

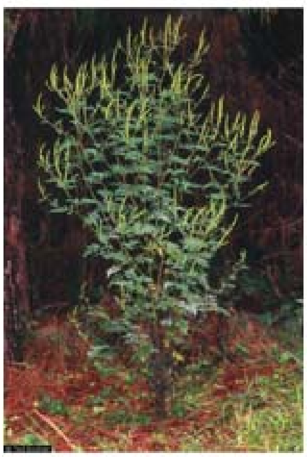
Duration: May – August
Frequency: One survey weekly
Time of day: 6 am – 11 am is ideal (some flowers may close during the heat of the day)
Weather: Do not survey during measurable precipitation events.
Study plots: Bryan Farms –NWSG plots, switchgrass monoculture plots, CP33 buffers,
Prairie Experiment Station - NWSG plots

Methods:

1) Prior to starting record plot id, date, observer. **DON'T FORGET THE DATE!**
2) Choose random starting point along plot edge.
3) At a reasonable brisk pace walk in a straight line (transect) through the center of the plot to the opposite edge.
4) Circle species observed in bloom (i.e., visible flower) as walking through the plot. If species is identified in bloom that is not listed, record it in the "Additional species in bloom" section at the bottom. Refer to the pictures below and field guides to help you confirm identification.
5) Choose another random starting point that does not overlap visual range of previous transect and repeat steps 3-4.
6) Travel to next plot and repeat.

# Bloom-time Survey Pollinator Plant List

**Partridge pea**
**(*Chamaecrista fasciculata*)**



**Annual ragweed**
**(*Ambrosia artemisifolia*)**

**Great ragweed**
**(*Ambrodia trifida*)**

**Canada goldenrod**
**(*Solidago canadensis*)**

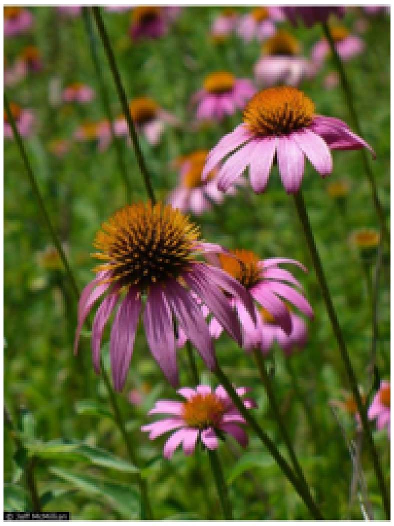
**Illinois bundleflower**
**(*Desmanthus illinoensis*)**

**Tickseed Sunflower**
**(*Bidens aristosa*)**

**Black-eyed susan**
**(*Rudbeckia hirta*)**

**Maximillian's sunflower**
**(*Helianthus maximiliani*)**

**Butterfly milkweed**
**(*Asclepias tuberosa*)**



**Purple coneflower**
(*Echinacea purpurea*)

**Greyheaded coneflower**
(*Ratibida pinnata*)

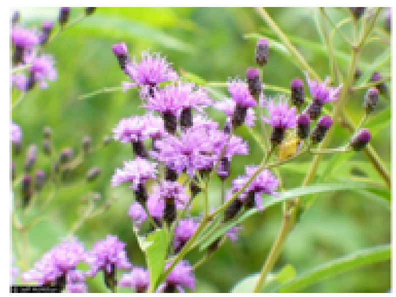
**Showy ticktrefoil**
(*Desmodium canadense*)

**Roundheaded lespedeza**
(*Lespedeza capitata*)

**Purple prairie clover**
(*Dalea purpurea*)

**Early buttercup**
(*Ranunculus fascicularis*)

**Brazilian vervain**
(*Verbena brasiliensis*)



**Giant ironweed**
(*Vernonia gigantea*)

**Horsetail**
(*Conyza canadensis*)

**Southern dewberry**
**(*Rubus trivialis*)**

**Sesbania (Sicklepod)**
**(*Sesbania spp.*)**

# Bloom-time Survey
# Weekly Data Collection Sheet

**Date:** **Plot:** **Observer:**

**Circle species in bloom:**

| | | | |
|---|---|---|---|
| **Partridge pea** (*Chamaecrista fasciculata*) | **Annual ragweed** (*Ambrosia artemisifolia*) | **Great ragweed** (*Ambrodia trifida*) | **Canada goldenrod** (*Solidago canadensis*) |
| **Illinois bundleflower** (*Desmanthus illinoensis*) | **Tickseed Sunflower** (*Bidens aristosa*) | **Black-eyed susan** (*Rudbeckia hirta*) | **Maximillian's sunflower** (*Helianthus maximiliani*) |
| **Butterfly milkweed** (*Asclepias tuberosa*) | **Purple coneflower** (*Echinacea purpurea*) | **Greyheaded coneflower** (*Ratibida pinnata*) | **Showy ticktrefoil** (*Desmodium canadense*) |
| **Roundheaded lespedeza** (*Lespedeza capitata*) | **Purple prairie clover** (*Dalea purpurea*) | **Early buttercup** (*Ranunculus fascicularis*) | **Brazilian vervain** (*Verbena brasiliensis*) |
| **Giant ironweed** (*Vernonia gigantea*) | **Horsetail** (*Conyza canadensis*) | **Southern dewberry** (*Rubus trivialis*) | **Sicklepod** (*Sesbania spp.*) |

**Additional species observed in bloom:**


**Date:** **Plot:** **Observer:**

**Circle species in bloom:**

| | | | |
|---|---|---|---|
| **Partridge pea** (*Chamaecrista fasciculata*) | **Annual ragweed** (*Ambrosia artemisifolia*) | **Great ragweed** (*Ambrodia trifida*) | **Canada goldenrod** (*Solidago canadensis*) |
| **Illinois bundleflower** (*Desmanthus illinoensis*) | **Tickseed Sunflower** (*Bidens aristosa*) | **Black-eyed susan** (*Rudbeckia hirta*) | **Maximillian's sunflower** (*Helianthus maximiliani*) |
| **Butterfly milkweed** (*Asclepias tuberosa*) | **Purple coneflower** (*Echinacea purpurea*) | **Greyheaded coneflower** (*Ratibida pinnata*) | **Showy ticktrefoil** (*Desmodium canadense*) |
| **Roundheaded lespedeza** (*Lespedeza capitata*) | **Purple prairie clover** (*Dalea purpurea*) | **Early buttercup** (*Ranunculus fascicularis*) | **Brazilian vervain** (*Verbena brasiliensis*) |
| **Giant ironweed** (*Vernonia gigantea*) | **Horsetail** (*Conyza canadensis*) | **Southern dewberry** (*Rubus trivialis*) | **Sicklepod** (*Sesbania spp.*) |

**Additional species observed in bloom:**

Appendix C. Effects of burning and disking on butterflies in native grass upland habitat buffers


**Acknowledgments**

Information in the note was adapted from research conducted by Jolie Goldenetz, graduate student in the Department of Wildlife, Fisheries and Aquaculture, Mississippi State University, under the supervision of Dr. Sam Riffell, Associate Professor of Wildlife Ecology and Management. Funding was provided by the NRCS Agricultural Wildlife Conservation Center, Mississippi Agricultural and Forestry Experiment Station (MAFES) and the College of Forest Resources. B. Bryan Farms and Prairie Wildlife allowed us to work on their land and implemented disturbances. The NRCS Mississippi State Office and the Clay County Farm Service Center provided technical assistance. This research was also supported by numerous graduate students in the Department of Wildlife, Fisheries and Aquaculture at Mississippi State.


**Suggested citation:**

Goldenetz-Dollar, Jolie, Sam Riffell, and L. Wes Burger, Jr. 2010. Effects of burning and disking on butterflies in native grass upland habitat buffers. U.S. Department of Agriculture, Natural Resources Conservation Service. Wildlife Insight # XXX.

**Cover photo**: Sachem Skipper in native grass field buffers in north-central Mississippi (*Photo by J. Goldenetz*)

# Effects of Burning and Disking on Butterflies in Native Grass Upland Habitat Buffers

## Introduction

Conservation buffers are narrow strips of land in agricultural landscapes which are maintained in permanent vegetation to achieve specific conservation benefits including soil erosion control, water quality enhancement and wildlife habitat. Upland habitat buffers are one type of conservation buffer specifically designed to provide breeding and wintering habitat for upland birds in agricultural systems. Upland habitat buffers are planted in native grasses and forbs or revegetated from natural succession. These buffers can be established under either Field Border (Code 386) or Early Successional Habitat Development (Code 647) practice standards and managed under Upland Habitat Management (Code 645) or Prescribed Fire (code 338) standards. Farm Bill conservation programs (e.g., Conservation Reserve Program, Wildlife Habitat Incentives Program, and Environmental Quality Incentives Program) provide financial and technical assistance for buffer design, installation and management, and numerous studies demonstrate how buffers intercept pollutants, reduce erosion and provide improved wildlife habitat. Landowners can meet multiple economic and ecological objectives with buffers through careful design, plant materials selection and management. Native grass buffers can also provide suitable habitat for pollinators including numerous butterfly species.

**Figure 1.** Native grass field buffer enrolled in CP33 Habitat Buffers for Upland Birds located in north-central Mississippi *(Photo by J. Goldenetz)*

Early successional plant communities are disturbance-dependent. Controlled, periodic disturbance treatments to revitalize plant cover are necessary for the long-term maintenance of native grass buffers. In the absence of disturbance over time, the forb component diminishes and grass cover increases. Also, a litter layer develops over time which creates a mechanical barrier to grass development and decreases the vigor of the grassland stand. This wildlife insight reports new information about how two common disturbance methods – prescribed burning and disking – influence butterfly communities.

**Figure 2.** Mid-contract management activities on native grass field buffers. Upper photo is fall disking, and lower photo is spring burning. *(Photos by J. Goldenetz*)

### Grassland butterfly communities

All butterflies require certain plant species - called host-plants - during their larval (caterpillar) life-stage. For example, a grassland species, Swarthy Skipper, consumes Little Bluestem (*Schizachyrium scoparium*) during its larval stage. Butterflies also require nectar sources - food-plants during their adult (flying) life-stage, which are primarily flowering forbs.

Grasslands support a suite of butterfly species that have co-evolved with native plants in prairie systems. These species (like the Swarthy Skipper) are restricted to grassland habitats, and some species may have specific grass species which they use as a larval host. Other species that use native grassland are butterflies are generalists found in a diverse array of habitats (from backyards to golf courses).

### Project description

### Study area

Bryan Farms is a privately-owned, 2,104-ha farm with row-crop and grazing operations located in the historical Blackland Prairie physiographic region of Northeast Mississippi. The area has a pre-settlement history of frequent fire (both natural and human-induced) and post-settlement history of agricultural intensification and fire suppression (Peacock and Schauwecker, 2003).

During spring 2004, 79 crop hectares were enrolled in CP33 Habitat Buffers for Upland Birds and planted with a seed mix containing species common in the Black Belt Prairie ecosystem: Big Bluestem (*Andropogon gerardii*), Little Bluestem, Indiangrass (*Sorghastrum nutans*), Partridge Pea (*Chamaecrista fasciculate*), Black-eyed Susan (*Rudbeckia hirta*), and Maximilian Sunflower (*Helianthus maximiliani*). The buffer plant community also included many non-seeded species which were present in the seedbank. The buffers surrounded fields planted in soybean, corn, or Bermuda grass.

### Experimental disturbances

Each field was randomly assigned to one of three treatments (i.e., fall disk, spring burn, and no management). One randomly-assigned buffer per field (i.e., one side of each field) received management (i.e., fall light disking or early spring burning) each year following a rotational management regime consistent with CP33 practice standards.

Light disking was used in this study because it promotes an early-successional plant community by cutting existing vegetation, incorporating at least half of the vegetation into the soil, and exposing a considerable about of soil (Greenfield et al., 2003). Disking occurred in fall because fall disking normally stimulates more desirable forbs, whereas disking at other times of the year may stimulate less desirable agronomic weeds such as Johnsongrass (Harper et al., 2007).

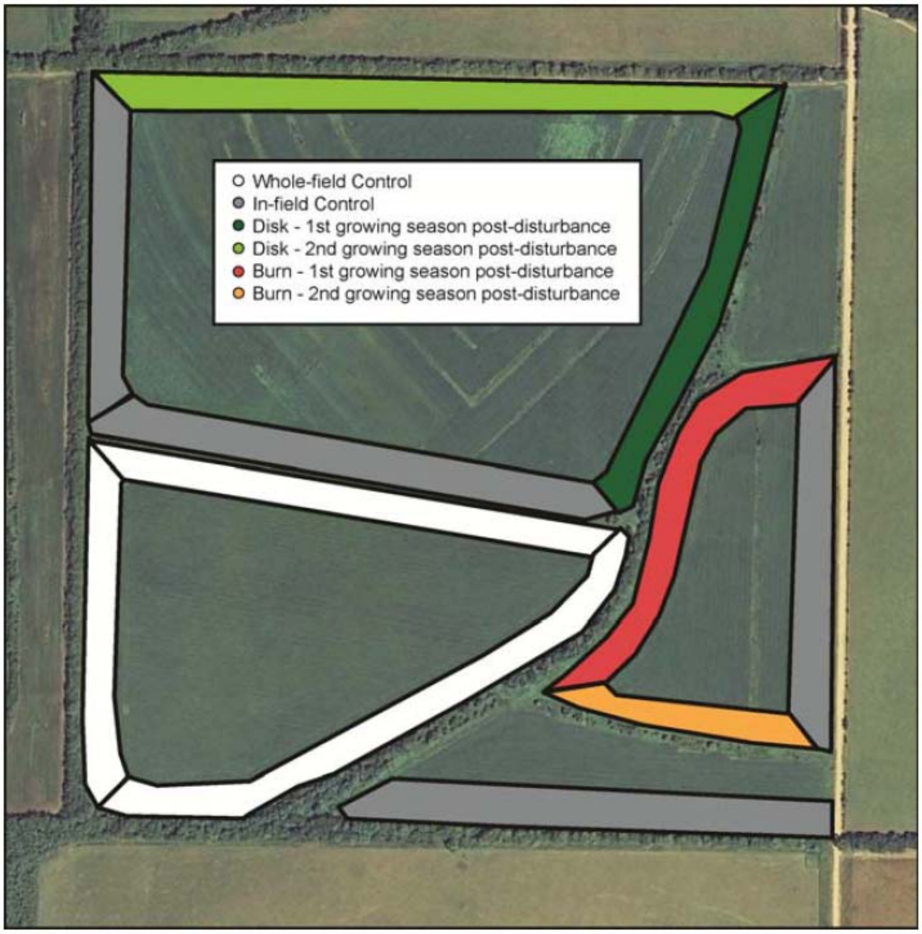



**Figure 3.** Experimental design for mid-contract management experiment. Disturbances were implemented 2007 – 2009. Color codes match those in Figures 4 and 5.

Prescribed burning was also used to maintain early-successional plant community structure, increased nutrient availability, and herbaceous growth stimulation; furthermore, burning in early spring (i.e., March-April) reduces winter cover for only a short period before spring green-up and does not disrupt wildlife nesting seasons (Harper et al., 2007). Also, an early spring burn produces less smoke (which can be negatively viewed by the public) compared to a burn after spring green-up has occurred.

### Butterfly sampling

To sample the butterfly community, we placed three 50-m transects in the center along the long axis of each buffer (Pollard and Yates 1993, and similar to those used by Ries et al. 2001 and Reeder et al. 2005). Having three separate transects per buffer helped prevent double counting of individuals (Swengel and Swengel, 1999). Along each 50-m transect, butterflies (including skippers) were counted six times each summer (June-August 2007-2009) from 8 am to 1 pm CST and favorable weather conditions (Ries et al., 2001).

**Essential results**

From 2007 – 2009, 45 species of butterflies were observed using native grass buffers. Fourteen species (12% of total observations) were grassland butterflies.

Planted native grass buffers in north-central Mississippi supported communities similar to those in other types of grasslands (Table 1) in terms of the proportion of the butterfly community comprised of grassland butterflies.

**Table 1.** Studies recording habitat-sensitive butterfly species and the percent of habitat-sensitive butterflies out of all detected butterflies.

| Study | Location | # Habitat-sensitive species and % of total individuals | |
|---|---|---|---|
| This study (2010) | Mississippi CP33 buffers | 14 | 12.0% |
| Shepherd and Debinski (2005) | Iowa remnant prairies | 6 | 9.6% |
| Reis et al. (2001) | Iowa roadside prairies | 10 | 10.0% |
| Reeder et al. (2005) | Minnesota filter strips | 11 | 15.0% |
| Vogel et al. (2010) | Iowa remnant prairie | 16 | 26.0% |

Disturbance tolerant butterfly species were more abundant and more species rich on disked buffers, most likely because disking promoted growth of forbs (Figure 4). Because 80% of butterflies were disturbance-tolerant, overall species richness and abundance were higher on disked buffers, too.

Grassland species abundance was lower the first year after a burn, but not significantly so. There were no other differences among treatments for grassland butterflies (Figure 5).

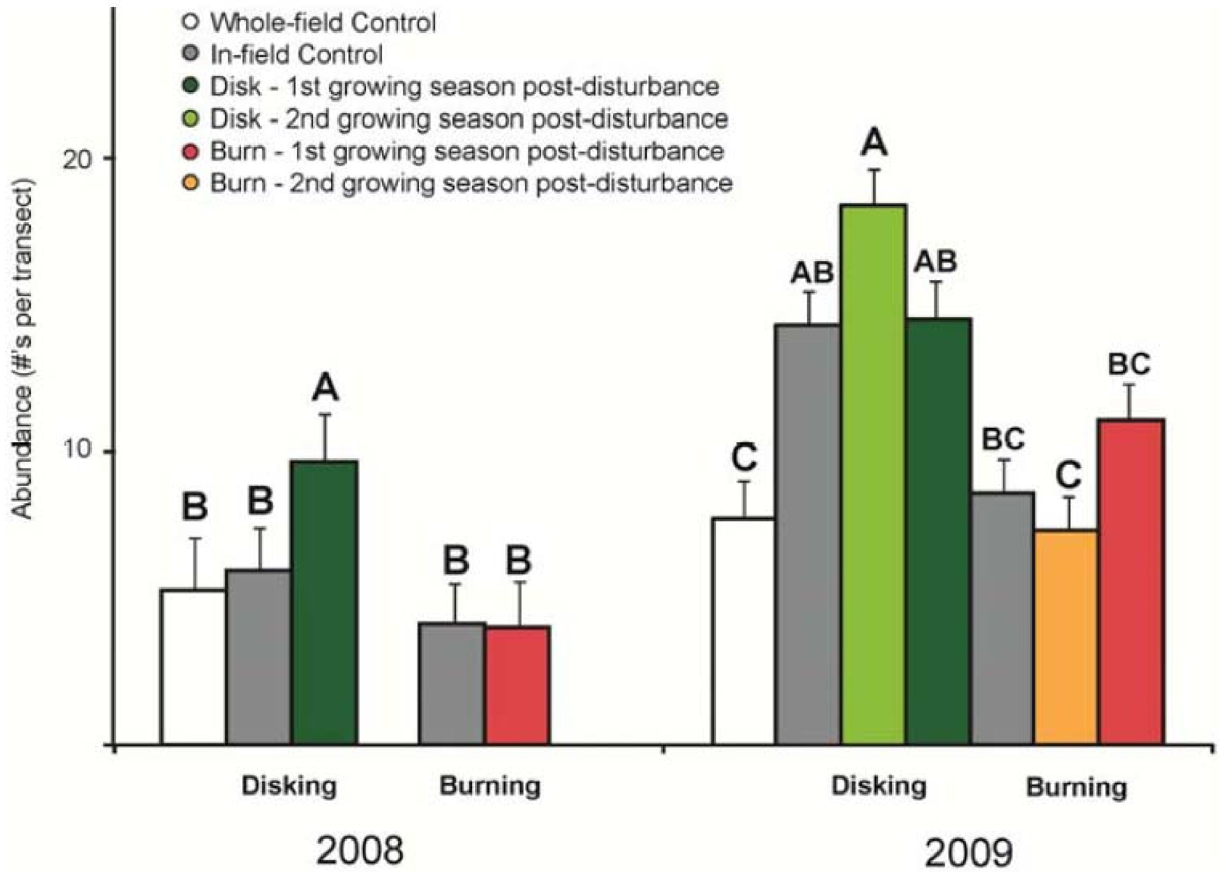



**Figure 4.** Mean total number of disturbance tolerant butterflies detected on native grass field buffers in the 1st and 2nd growing seasons after planned disturbances. Means with letters in common were not significantly different.

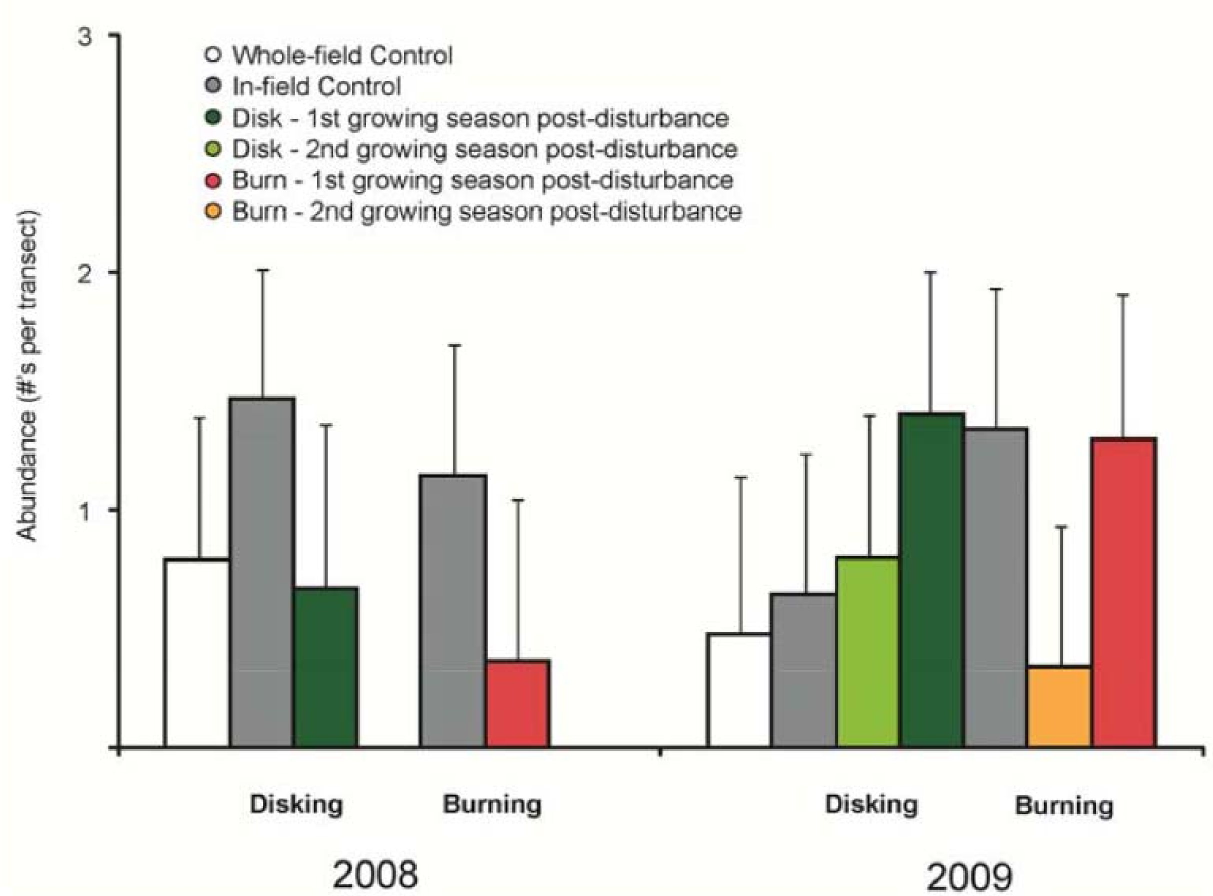



**Figure 5.** Mean total number of grassland butterflies detected on native grass field buffers in the 1st and 2nd growing seasons after planned disturbances. Means were not significantly different.

**Mid-contract management options**

Fire is generally considered a negative disturbance for butterflies because many overwintering larvae can be destroyed. Thus, non-fire refugia (like used in this experiment) are important considerations in a disturbance plan (Swengel and Swengel, 2006). These results indicate that the in-field and whole-field controls provided sufficient refugia habitat to maintain butterfly abundance and species richness. We caution that the recovery time of butterfly populations can vary greatly. In more northern settings, fire-intervals may need to be > 5 growing seasons to allow butterfly populations to completely recover (Vogel et al. 2010). In contrast, butterfly abundance was greatest in the 1$^{st}$ growing season post-fire in pine grasslands of Arkansas (Rudolph et al. 2006). The results of this experiment suggest that USDA practice standards that restrict mid-contract management to 1/3 or 1/4 of buffer area in a given year are sufficient to protect grassland butterflies in the Southeast. Regional standards should be developed for other grassland systems in other regions.

Disking also resulted in higher abundance and richness of disturbance-tolerant butterflies. In the Southeast, prescribed fire often presents logistical hurdles (e.g., weather restriction or lack of expertise) and perceived liability. In these situations, disking (when done on a rotational basis) is a viable alternative to prescribed fire that also does not negatively impact butterfly communities, although disking may shift butterfly community composition to higher proportion of disturbance-tolerant species. Moreover, disking is often easier to implement compared to burning in terms of equipment, licenses, and weather restrictions. In a logistically and economically ideal situation, land-owners could best enhance butterfly communities by implementing diverse combination of disking and burning (but only on 1/3 to 1/4 of buffer area per year).

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Ries, L., D.M. Debinski, and M.L. Wieland.2001. The conservation value of roadside prairie restoration to butterfly populations. Conservation Biology 15:401-411.

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Appendix D. Biofuels brochure.

## RECOMMENDATIONS FOR FUTURE RESEARCH

Research on wildlife in pine stands intercropped with switchgrass has shown that there is a strong influence based on the growing season after switchgrass was planted, the taxa being studied, the year, and the parameter of interest such as abundance or diversity. This study organized insects into guilds (pest, pollinator, and predator) and recorded them at the family level. It is possible, however, that research on insects at a genus and species level would produce different results. In addition, the trapping methods used in this research may have affected the type of insects collected. Using different trapping methods may show different results.

## COST-SHARE PROGRAMS

The FSA Biomass Crop Assistance Program (BCAP) provides financial assistance to owners and operators of agricultural and nonindustrial private forest land who wish to establish, produce, and deliver biomass feedstocks.



Natural Resources Conservation Service
www.nrcs.usda.gov

Mississippi State University
www.msstate.edu

Wildlife Mississippi
www.wildlifemiss.org

Mississippi Agricultural and Forestry Experiment Station
www.mafes.msstate.edu

Forest and Wildlife Research Center
www.fwrc.msstate.edu

Weyerhauser
www.weyerhaeuser.com

Photo by Jeff McMillian @ Almost Eden, hosted by the USDA-NRCS PLANTS Database

POLLINATOR SERIES

# BIOFUELS IN THE FOREST

Effects of Intercropping Switchgrass in Pine Stands on the Insect Community

Myung-Bok Lee, Joshua W. Campbell, James A. Martin, and Darren A. Miller

*Insects are an important component of biodiversity in forest ecosystems. They are a food source for birds and other wildlife. They help pollinate flowering plants. They can also affect the production of biofuels directly – either through feeding on the crop or helping to control the population of harmful insects. Maintaining the community of insects in an intensively managed forest environment is important to ensuring the sustainability of the system.*

Sweat bee

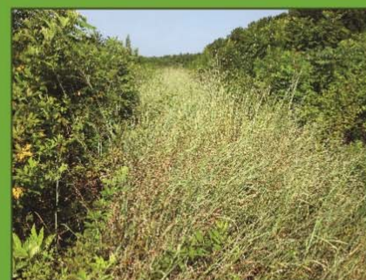
## SWITCHGRASS & BIODIVERSITY

In intensively managed pine stands, growing switchgrass yields ecological benefits by providing cover and nesting areas for insects and wildlife. Switchgrass can also help to capture carbon and control soil erosion. However, planting switchgrass changes the composition of plants under the trees and, as a result, may change the diversity of animals and insects.

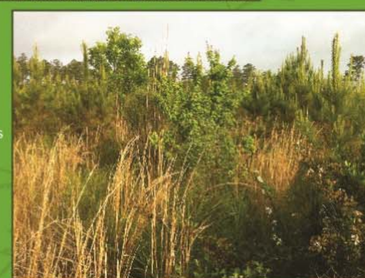
## SWITCHGRASS IN FORESTS

Switchgrass (Panicum virgatum L.), a perennial, warm-season native grass found throughout the majority of the United States, can be grown to produce biofuel. It can grow in a variety of soil and moisture conditions. It is relatively easy to establish, grows quickly, and can produce a large amount of biomass.

Growing two or more crops in proximity to each other (intercropping) can maximize profits and yields. In pine stands, switchgrass can be planted between the rows of trees and harvested semiannually or annually until the pines shade out the switchgrass.



Intercropped young stand (>4 years old)



Control young stand (>4 years old)

## STAND AGE & INSECTS

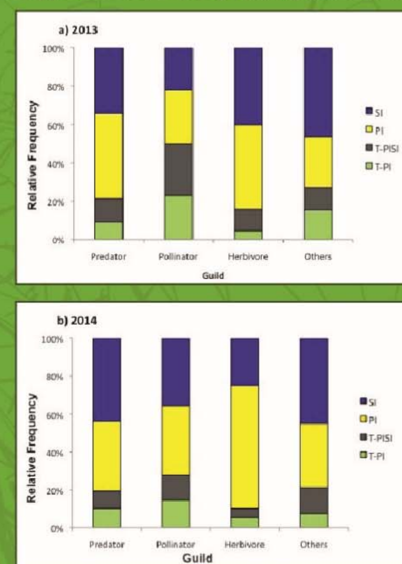
As a pine stand gets older, the tops of the trees (the tree canopy) spread wider and put on more branches and needles. This growth reduces the amount of sunlight that can reach the forest floor. In a young pine stand, the trees are smaller and have fewer branches and needles, so more light reaches the forest floor, resulting in a diverse community of plants, wildlife, and insects under the trees. As the pines age and the canopy closes, there are typically fewer plants and less plant diversity under the trees. As the plant community changes, the diversity of wildlife and insects found under the forest canopy also changes.

## THE STUDY

Researchers at Mississippi State University (MSU) studied the effects of growing switchgrass in intensively managed pine plantations on the insect community on plots established by Catchlight Energy, LLC on land owned and managed by Weyerhaeuser Company. They collected insects in young stands (>4 years old) and mid-rotation stands (~10 years old), both with and without switchgrass planted among the trees. They then looked for differences in the types and diversity of insects found in each stand type.

## EFFECTS ON INSECTS

Planting switchgrass in intensively managed pine stands did not significantly alter the insect community at the family level. The abundance, diversity, and composition of the insect community was much more strongly affected by stand age than by intercropping.




Relative proportion of abundance of insect foraging guilds at each practice. "Predator" represents predators/parasites/parasitoids foraging guild. Treatment: T-PI, 10-year old stand with no intercropped switchgrass; T-PISI, 10-year old stand with intercropped switchgrass; PI, 5-year old stand with no intercropped switchgrass; SI, 5-year old stand with intercropped switchgrass.

Appendix E. Butterflies brochure.

## QUICK TIPS

For best results, implement a diverse combination of disking and burning to enhance butterfly communities.

Follow the USDA practice standards restricting mid-contract management activities to 1/3 or 1/4 of the buffer area in a given year.

There may be a change in the species of butterflies using the buffer after disking or burning, so management activities intervals need to be long enough to allow butterfly populations to recover.

## COST-SHARE PROGRAMS

Farm bill conservation programs provide financial and technical assistance for buffer design, installation, and management, learn more at: http://www.nrcs.usda.gov/wps/portal/nrcs/main/national/programs/farmbill/

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Natural Resources Conservation Service
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Mississippi State University
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www.mafes.msstate.edu

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www.fwrc.msstate.edu

MAFES NRCS Wildlife Mississippi
MISSISSIPPI STATE UNIVERSITY
College of Forest Resources
Forest and Wildlife Research Center

POLLINATOR SERIES

# BUTTERFLIES
## IN CONSERVATION BUFFERS

Effect of Management Activities on Butterflies in Native Grass Conservation Buffers

Jolie Goldenetz-Dollar, Sam Riffell, and L. Wes Burger, Jr

*Butterflies help pollinate many native grassland plants and serve as food to other insects and birds. They are important components of biodiversity in grassland ecosystems. As the intensity of agricultural production has increased across North America, native grassland habitats are disappearing in the working landscapes.*

## CONSERVATION BUFFERS

Conservation buffers are narrow strips of land maintained in permanent vegetation that are established at the edges of row crop fields that are designed to intercept pollutants, reduce erosion, and provide improved habitat for wildlife.

Native grasses and flowering plants are prescribed in the United States Department of Agriculture's (USDA) Conservation Reserve Program practice CP33 Habitat Buffers for Upland Birds. The recommended management activities to maintain these buffers include light disking and prescribed fire. Butterfly host and food plants are affected by these management activities.

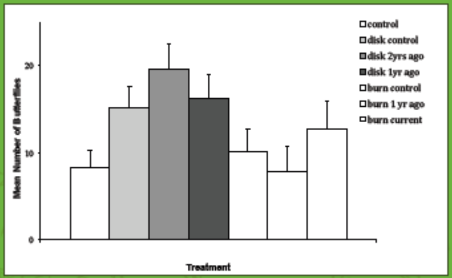
## MANAGEMENT ACTIVITIES

Light disking helps to promote the grass and flowering plant communities desirable in these buffers. It disturbs the existing vegetation, incorporating at least half of it into the soil, and exposes a considerable amount of soil at the same time. Disking in the fall encourages more desirable flowering plants and discourages weeds.

Prescribed fire also maintains the desirable plant communities in buffers by increasing nutrient availability and stimulating the growth of herbaceous plants. Burning is done in the early spring to reduce winter cover for a short period of time before spring green-up. Burning during this time also reduces the amount of smoke produced compared to burning later in spring.

## EFFECTS ON BUTTERFLIES

Planted native grass conservation buffers are home to many of the same butterflies as natural grasslands, so maintaining these communities at the edges of agricultural fields increases diversity across the landscape. Neither of the two recommended management methods negatively impacted the butterfly communities when a portion of the buffer was left undisturbed to protect butterfly larvae




PESCRIBED FIRE: Fire can destroy overwintering butterfly larvae, so it is important to leave a portion of the buffer unburned to maintain butterfly populations. The time it takes for butterfly populations to recover from fire varies regionally. In the first year after a burn, more of the butterflies that can tolerate the disruption in their habitat are present than usual.

DISKING: As with burning, more of the butterflies that can tolerate the disruption in their habitat are present than usual in the first year after disking. Disking is a viable alternative to prescribed fire.

USDA practice standards restrict these management activities to 1/3 or 1/4 of the buffer area in a given year; the unmanaged portion of the buffer acts as a refuge for butterfly species during disking or burning activities. This allows butterfly populations to recover throughout the buffer.

Appendix F. Bloom time chart.

# FLOWERING PLANTS
## IN SEMI-NATURAL GRASSLANDS
Bloom Times and Insect Pollinators

POLLINATOR
SERIES

# TOP 9 FLOWERING PLANTS
## RECOMMENDED BY NRCS
## FOR PLANTING IN CONSERVATION PRACTICES

Butterfly milkweed *Asclepias tuberosa*
Bearded beggarticks *Bidens aristosa*
Partridge pea *Chamaecrista fasciculate*
Purple prairie clover *Dalea purpurea*
Illinois bundleflower *Desmanthus illinoensis*

Purple coneflower *Echinacea purpurea*
Maximilian sunflower *Helianthus maximiliani*
Pinnate prairie coneflower *Ratibida pinnata*
Black-eyed Susan *Rudbeckia hirta*

| PLANT SPECIES[1] | BLOOM TIME[2] | | | | | | MOST COMMON POLLINATORS[3] |
|---|---|---|---|---|---|---|---|
| | MAY | JUN | JUL | AUG | SEP | OCT | |
| Purple coneflower | ■ | | | | | | Butterflies, bees |
| Purple prairie clover | ■ | | | | | | Honey bees, leafcutter bees |
| Black-eyed Susan | ■ | ■ | | | | | Bees, flies |
| Maximilian sunflower | ■ | ■ | ■ | | | | Butterflies, bees, wasps, flies, beetles |
| Illinois bundleflower | ■ | ■ | ■ | ■ | ■ | | Skippers |
| Butterfly milkweed | | ■ | ■ | | | | Butterflies, bees, wasps |
| Pinnate prairie coneflower | | ■ | ■ | | | | Butterflies, bees, wasps, flies, beetles |
| Bearded beggarticks | | ■ | ■ | ■ | ■ | ■ | Sweat bees, bumble bees, swallowtail butterflies |
| Partridge pea | | ■ | ■ | ■ | ■ | ■ | Bumble bees, honey bees, leafcutter bees |

*1 These flowering plants are generalists when attracting pollinators and observed throughout conservation buffers (studied in Mississippi).*
*2 Bloom time of each species in the table is limited by the type of conservation practice applied. Some of those species can bloom for a longer period of time.*
*3 This is a list of the pollinators most commonly seen at the plants during the research period.*

# 5 COMMON FLOWERING PLANTS

## FOUND IN AND AROUND ROW CROP FIELDS

Asters
*Symphyotrichum patens/pilosum*

Goldenrod
*Solidago canadensis*

Brazilian vervain
*Verbena brasiliensis*

Daisy fleabane
*Erigeron annuus*

Late boneset
*Eupatorium serotinum*

| PLANT SPECIES[1] | BLOOM TIME[2] | | | | | | MOST COMMON POLLINATORS[3] |
|---|---|---|---|---|---|---|---|
| | MAY | JUN | JUL | AUG | SEP | OCT | |
| Brazilian vervain | ■ | ■ | ■ | ■ | ■ | ■ | Swallowtails and other butterflies |
| Aster | | ■ | ■ | ■ | ■ | ■ | Butterflies, bees |
| Daisy fleabane | | ■ | | | | | Sweat bees, skippers, hoverflies |
| Goldenrod | | | | | ■ | ■ | Bees, butterflies, wasps, beetles |
| Late boneset | | | | | | ■ | Bees, butterflies, wasps, beetles |

*1 These flowering plants are generalists when attracting pollinators and observed throughout conservation buffers (studied in Mississippi).*
*2 Bloom time of each species in the table is limited by the type of conservation practice applied. Some of those species can bloom for a longer period of time.*
*3 This is a list of the pollinators most commonly seen at the plants during the research period.*

Authors: James Martin, Myung-Bok Lee, and Josh Campbell
Flower images from the LadyBird Johnson Wildflower Center at www.wildflower.org

MISSISSIPPI STATE UNIVERSITY
College of Forest Resources Forest and Wildlife Research Center
MAFES Mississippi Agricultural & Forestry Experiment Station
NRCS
Wildlife Mississippi

Appendix G.  Effect of bioenergy crop type and harvest frequency on beneficial arthropods

# Effect of bioenergy crop type and harvest frequency on beneficial arthropods

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## Abstract

Switchgrass (*Panicum virgatum* L.) and a mix of native warm season grasses with forbs (NWSG-mix) have received great attention as cellulosic biofuel sources. While numerous studies support greater biodiversity at those biofuel crops than corn, it is unclear how switchgrass and NWSG-mix affects beneficial arthropods, pollinators (PO) and predators/parasites/parasitoids (PPP). Few empirical studies have also explored the effects of biofuel crop harvest on arthropods. We investigated the responses of PO and PPP insects to those two biofuel crops and harvest frequency in Mississippi, USA, during 2011-2013. We established 16 fields on agricultural lands and randomly assigned each one of 4 treatments: switchgrass with single harvest; switchgrass with multiple harvests; NWSG-mix with single harvest; NWSG-mix with multiple harvests. We set up 3 sampling stations per field and conducted insect sampling 1-3 times monthly during summer to fall using colored bowl traps. We tested treatment effects with a general linear mixed model, Tukey's HSD test, and redundancy analysis. Biofuel crop type significantly influenced abundance and Family diversity, whereas effects of harvest frequency were insignificant. Abundance was high in switchgrass during summer and in NWSG-mix during fall. Family richness was greater in NWSG-mix. Most Families did not show a strong association with treatments. Our results suggest that 1) biofuel crop type is a more important factor influencing beneficial insects than harvest frequency and 2) switchgrass provide more resources for insects during summer when most insects are abundant, however, NWSG-mix can be more appropriate to enhance insect diversity for a longer period of time.

Key words: abundance, diversity, haying, native warm season grass, perennial prairie grass, switchgrass

## Introduction

With growing demand for energy independence and reduction in carbon emissions, interest in biofuels has increased in North America and Europe. In USA, the Energy Independence and Security Act of 2007 calls for 36 billion gallons of renewable fuels being domestically produced by 2022 and 16 billion gallons of the fuels from cellulosic sources [1]. While corn (*Zea mays* L.) is currently used as the main biofuel crop in USA, using corn and other annual food crops for biofuel feedstock raises concerns about possible inflation of food prices, increase in nutrient (e.g., fertilizer) input, and reduction in air and water quality [2-4]. To avoid these economic and environmental issues, there is growing interest in using perennial prairie grasses such as switchgrass (*Panicum virgatum* L.), big bluestem (*Andropogon gerardii*), and Indiangrass (*Sorghastrum nutans*) as alternative crops. In particular, switchgrass has been extensively studied due to its high biomass yield potential and broad adaptability to a wide range of environmental conditions [5-9].

Perennial prairie grasses have also received great attention from wildlife ecologists and conservation biologists because they may reduce the conflict between biofuel production and biodiversity conservation by land sharing or sparing land [4, 10-11]. Most perennial prairie grasses considered for biofuel crops are native to the North American tall-grass prairie and known as native warm season grass (NWSG). In several Conservation Reserve Programs, they are used to restore early succession/grassland habitats or planted at field margins to mitigate negative influences of agricultural land use on biodiversity. [10, 12-13].

A number of recent studies support significantly greater diversity or abundance of arthropods, birds, and plants in switchgrass monoculture and polycultures (e.g. NWSGs in mixture or mixed-NWSGs-forb prairie) than in corn monoculture [11, 14-17]. Although perennial prairie grasses produce lower biomass yields compared to corn monoculture, they provide a greater array of ecosystem services including pest control, pollination services, and wildlife habitat [17]. Mixed-NWSGs-forb prairie (NWSG-mix, hereafter) planting also requires low inputs for establishment and maintenance [18]. It is hypothesized that NWSG-mix planting may increase biodiversity more than switchgrass monoculture because diverse plants could create spatially and temporarily heterogeneous habitats that can harbor a variety of species [10]. Several arthropod studies tested this hypothesis but their findings varied by study. Robertson et al. [16] supported the hypothesis, whereas Gardiner et al. [14] did not find significant differences. Thus, the relative effects of potential NWGS biofuel crop types (switchgrass planting vs. NWSG-mix planting) on arthropod community remains unclear. In addition to the crop types, biofuel crop management such as harvest frequency and timing of harvest can influence biodiversity [19]. For instance, single harvest (one cut during fall) or multiple harvests over years is expected to improve wildlife habitat value by providing important resources or habitat conditions for a wide range of bird species, compared to multiple harvests within a year [10]. However, this is rarely tested, particularly with arthropods [20].

Arthropods are key providers of ecosystem services including pest control and pollination and main food sources for breeding birds [21-23]. The annual value of ecosystem services provided by beneficial insects is estimated to be $ 8 billion in USA and at least $57 billion if recreational benefits are included [24]. Arthropods can also affect biomass and crop yields as well as establishment of biofuel crops negatively or positively by acting as pests, predators, or decomposers [22, 25-26]. Given their importance to agroecosystems, understanding relationships between arthropods and potential biofuel crop types and crop management is critical to develop management regimes minimizing biodiversity loss while maintaining benefits from ecosystem services and biomass production. In this study, we report how insect pollinators and other beneficial insects' (predators/parasites/parasitoids) abundance, familial diversity, and composition respond to NWSG biofuel crop types (switchgrass and NWSG-mix) and harvest frequency (single harvest and multiple harvests). We also consider possible seasonal variation in the responses of those two guilds, which is less examined in previous studies.

**Methods**

1. Study site and treatment

The study was performed at the Bryan Farms in Clay County, Mississippi, during 2011-2013. The study area is part of the Blackland Prairie where the predominant land use is timber production (44%), livestock production (20.81%), and small fields of soybeans and corn (28.39%). Bryan Farms encompasses over 5400 acres of agricultural land and approximately 25% of the land base has been allocated to a myriad of conservation treatments under a diversity of Conservation Programs including Environmental Quality Incentive Program, Wildlife Habitat Incentive Program and Conservation Reserve Program.

At Bryan Farms, we established four experimental blocks composed of 4 fields previously under soybean production, resulting in a total of 16 fields. All fields were closely located and 18-20 acres in size except two fields (12.5 acres). Within a block, we assigned randomly each of the following four treatments associated with biofuel crop type and harvest frequency to each field: (1) NativeM, NWSG-mix with a single biomass harvest; (2) NativeS, NWSG-mix with multiple harvests to simulate haying and biomass collection; (3) SwitchM, Switchgrass monoculture with multiple harvests to simulate haying and biomass collection; (4) SwitchS, Switchgrass monoculture with a single biomass harvest. NWSG-mix included a mix of big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium soparium*), Indiangrass (*Sorghastrum* *nutans*), and selected prairie forbs (see Appendix 1 for the list of forbs). All grasses were planted in spring 2010 and harvest did not occur until 2012 to ensure establishment of grasses. In 2012, the first cut (dormant harvest) was applied to all fields in early April and the second cut (summer harvest) to SwitchM and NativeM fields in late June to simulate multiple harvests. SwitchM and NativeM also received one more cut in between late June and early July, 2013. Although the first harvest was planned in January 2012, it could not be performed due to unstable ground conditions caused excessive rainfall.

2. Insect collection

We used colored pan traps (or bowl traps) for insect sampling. We prepared three 12 oz bowls and each of them was colored differently with blue, white, or yellow as one set of traps. Those bowls were filled with a soap-water solution. We placed one set of bowls to the height of flowers or vegetation if forbs have not begun to flower at three locations (sampling station, hereafter) within a field. Sampling stations were spaced > 25m from the edge of a field to avoid edge effects and ≥ 50m from the nearest station to minimize dependency between trap sets. A total of 48 sampling stations (3 stations x 16 fields) were established across study fields.

To account for seasonal variations in insect populations, we performed trapping each month during May-November in 2011, May-October in 2012, and June-October in 2013. One set of bowl traps were installed at each sampling station twice (about 10-14 days apart) a month except May, August, September and November in 2011 (one sampling occasion), September and October in 2012 (one sampling occasion), and August in 2012 (three sampling occasions). Sampling occasions varied because some sampling events were lost from extreme wind or rain storms. Contents of each trap were collected three days following trap set up. Collected insect samples were preserved in a 70% ethanol solution for future identification.

3. Data analyses

While insect sampling was performed at all 16 fields, two fields (1 SwitchM and 1 SwtichS) established poorly. We excluded the data collected at those two fields and used the data from 14 fields, 42 sampling stations, for analysis.

Among insects captured, we focused on insects of two foraging guilds, pollinators (PO) and predators/parasites/parasitoids (PPP), due to their importance in the provision of ecosystem services. Those beneficial insects were counted and identified to Family. Families belonging to Lepidoptera were considered pollinators given that all Lepidoptera trapped were adults and butterflies (and moths in some cases) are often recognized as important pollinators.

To examine effect of treatment on insect abundance and insect diversity at Family level, we used abundance (number of individuals) of beneficial insects (TOTAL, sum of PO and PPP), abundance of PO and of PPP, Family richness (S, number of Families), Shannon-Wiener diversity index (H) as response variables. We conducted a separate analysis for each month and used mean values of response variables (abundance variables and diversity-related variables, S and H) per sampling station during each month. Abundance variables were log(x+1)-transformed prior to analysis to avoid biases because of high abundances of some Families. For diversity-related response variables, we did not include Families detected at <4 traps (~ <10% of total number of traps) to minimize biases due to the occurrence of uncommon families. We examined homogeneity of variance assumption using Levene's test. When violation of homogeneity assumption was found, we allowed different variances among treatments in the model ("varIdent" function, [27]). Significance of treatment effects were tested using a general linear mixed model with treatment as a fixed effect and field as a random effect because we considered a sampling station as a unit for analysis and sampling stations were nested within fields. We also conducted Tukey's HSD test for multiple pair comparisons when significant effect ($P$<0.05) was found. All of these analyses were performed in R with package "nlme" [28] and "multcomp" [29]. Diversity-related variables were also calculated in R with package "vegan" [30].

The relationship between the treatments and structure of insect community was investigated using analysis of similarity (ANOSIM) and redundancy analysis (RDA). ANOSIM tests whether there is a significant compositional difference between groups by comparing distances between groups with distances within groups. Bray-Curtis similarity was used as a measure of distance. The value of ANOSIM statistic R ranges 1 to -1, indicating high similarity among groups (more than within a group) to high dissimilarity among groups and no relationship when R=0. RDA which is commonly used for the analysis of community composition data is a combination of regression analysis and principal component analysis [31]. We pooled all monthly data and performed analysis by year. We used mean log(x+1)-transformed abundance of each family. ANOSIM and RDA were carried out in R with package "vegan".

## Results

1. General pattern

Over 98,600 individuals from 23 Families of beneficial insects were trapped during 2011-2013 (Appendix 2). Total number of insects captured was similar between 2011 (39,279) and 2012 (39,267), but lower in 2013 (20,119). The number of Families observed were similar between years: 21, 20, 19 families in 2011, 2012, and 2013, respectively. Among the 23 Families, Dolichopodidae and Halictidae were the most dominant all years, comprising 88- 94% of total insects during 2011-2013. While 20 families were captured at >10% of total sampling stations across 3 years, 3 families (Nymphalidae, Scoliidae and Sphingidae) were captured at less than 5% of total sampling stations.

2. Effect of treatment

*Abundance*

A significant difference in abundance of beneficial insects (TOTAL) among treatments was found in June and November, 2011 and October, 2012 (Table 1). All of the significant effects were observed between two treatments associated with biofuel crop type, NWSG-mix treatment (NativeM and NativeS) and switchgrass treatment (SwitchM and SwitchS) (Fig. 1). Conversely, abundance of TOTAL was not significantly different within NWSG-mix or switchgrass treatments, i.e., between single harvest and multiple harvests, suggesting little influences of harvest frequency on insect abundance. Although seasonal variations in abundance among treatments were not clear due to no significant responses in 2013, abundance of TOTAL tended to be higher at switchgrass treatments during summer (June, 2011) and at NWSG-mix treatments during fall (November and October in 2011 and 2012, respectively).

Pollinators (PO) showed patterns similar to TOTAL in 2011 and 2013 (Table 1 and Appendix 3). However, abundance of PO was also significantly different between NativeM and NativeS during November 2011 when no harvest occurred, indicating potential variations that may not be related to the treatment of harvest frequency among fields planted with NWSG-mix. This pattern was also observed in the abundance of predators/parasites/parasitoids (PPP) during July 2011 (Appendix 3). Like TOTAL, abundance of PO and of PPP were lower in NWSG-mix treatments during summer, 2011 and higher during fall, 2012 (PPP) and 2013 (PO). PO and PPP did not show significant responses to treatments in 2012 and 2013, respectively (Table 1).

*Family diversity*

Family richness (S) was also influenced by treatments during October in 2011, Jun-July in 2012, and August-October in 2013 (Table 2). Like the pattern observed in abundance, most significant responses were found between two treatments of biofuel crop types, NWSG-mix and switchgrass treatment (Fig. 2). Richness tended to be greater at NWSG-mix treatments than switchgrass treatments in 2011 and 2013. In 2012, Family richness was significantly greater at NativeS than at NativeM during June before the second cut, indicating potential variations unrelated to the treatment of harvest frequency between NativeS and NativeM fields. No other significant difference was observed between single harvest and multiple harvests treatment within the same biofuel crop type.

Shannon-Wiener diversity (H) was also significantly different between NWSG-mix treatments and switchgrass treatments (Table 2 and Appendix 4). The values of H were higher at NativeM during August, 2011 and 2013, whereas it was significantly higher at switchgrass treatments during October in 2012 and June in 2013. There were no significant differences in H between single harvest and multiple harvests.

*Composition of insect community*

The relative proportion of PO was higher at NWSG-mix treatments (40% at NativeM and 33%) at NativeS than in switchgrass treatments (25%) in 2011, but showed 7-10% decline at NWSG-mix treatments in 2012. In switchgrass treatments, PPP composed ~75% of the beneficial insect community and its relative proportion did not change between 2011 and 2012. In 2013, the beneficial insect community in all treatments was dominated by PPP (≥ 90%).

Dissimilarity in the composition of PO and PPP Families was weak among treatments: ANOSIM statistic R = 0.19 (p = 0.001), 0.11 (p = 0.001) and 0.18 (p = 0.001) in 2011, 2012 and 2013, respectively. The results of RDA also showed similar patterns: treatment explained only 26.5% of the total variances in the family assemblage in 2011, 19.4 % in 2012, and 21.3% in 2013. In 2011, the first canonical axis of RDA tended to separate NativeM from other treatments and the second axis distinguished NativeS from others, which supports the pattern of significant differences in abundance within NWSG-mix treatments (Fig. 3). Single harvest treatment was separated from multiple harvests treatment in 2012 and switchgrass treatment from NWSG-mix treatment in 2013 along the first axis. More Families tended to be associated with NWSG-mix treatments in 2011 and particularly 2013, and with single harvest treatments in 2012. During 2011-2013, Syrphidae (S4) was consistently positively related to switchgrass treatment (especially, SwitchM), whereas Halictidae (H1) and Hesperidae (H2) to NWSG-mix treatments. Apidae also showed a positive correlation with NativeM. Dolichopodidae (D1) was associated with SwitchS in 2011, NativeS in 2012, and both NativeS and NativeM in 2013. However, overall, over half of families analyzed were located at the center of the biplot, showing unclear correlations with treatments.

## Discussion

Our results show that biofuel crop type does have a profound impact on insect community as observed in previous studies. Most significant effects of treatments on total insect and guild-level abundance and Family diversity were found between switchgrass plantings and NWSG-mix plantings. A number of studies have reported greater abundance of insects, particularly, pollinators and predators as well as greater species richness of plants, birds, and insects at perennial native grass (largely, switchgrass) and NWSG-mix (mixed-NWSGs-forb) biofuel crops compared to a dominant biofuel crop, corn [11, 14, 16-17, 26]. However, the relative effect of switchgrass monoculture and NWSG-mix polyculture is inconsistent among taxon and studies. For example, Gardiner et al. [14] observed no significant differences in bee abundance and richness between prairie sites and switchgrass sites. However, they pointed out the possibility of low bee abundance in switchgrass sites where they are entirely managed for biofuel feedstock. While Werling et al. [17] observed the same pattern in richness of bees and birds, they also found substantially greater richness of plants, herbivorous insects, and predator insects at prairie sites. Although the findings of empirical studies are variable, it is generally hypothesized that biofuel crops composed of a mix of perennial native grasses and forbs can support more species by increasing habitat heterogeneity and providing a wide range of resources for diverse species compared to switchgrass monocultures [10]. Our study supports this hypothesized pattern given that greater richness (2011 and 2013) and several cases of higher Shannon-Wiener diversity (August in 2011 and 2013) at NWSG-mix plantings as well as the positive associations of more Families with NWSG-mix treatments (2011 and 2013, RDA results). Lower Shannon-Wiener diversity at NWSG-mix plantings during October 2012 and June 2013 may be considered contradictory. However, one should note that this is due to skewed distribution of abundance caused by Dolichopodidae. Although richness was greater at NWSG-mix plantings, log (x+1) transformed mean abundance of Dolichopodidae was 4-25 times higher compared to that of most other Families at NWSG-mix plantings (particularly, NativeS), whereas 3-6 times higher at switchgrass plantings.

Unlike the responses of Family richness, abundances of insects showed different patterns depending on season. This is noticeable because sampling periods of previous studies are limited to one season, summer or late-spring to summer, when most insect activities and vegetation growth are at their peak. Potential seasonal variations in the responses of insects are not often examined. In our study, switchgrass plantings tended to harbor more insects during early- to mid-summer, whereas abundance of insects was higher at NWSG-mix plantings during fall. This indicates that NWSG-mix polycultures could increase temporal heterogeneity due to possible dissimilarities in the time of growth and bloom among plants composing NWSG-mix and provide resources (nest sites, foods, shelters, etc.) for insects for a longer period of time. Conversely, amount of resources available for insects could be higher at switchgrass monoculture which would show synchronous growth during late spring-summer.

In contrast to the significant effects of biofuel crop types, harvest frequency showed marginal influences on insects. Based on RDA results, more families tended to be correlated with single harvest treatments in 2012. However, overall explanatory power by harvest frequency was low (14.7%). In addition, we did not find significant differences in abundance and family diversity between single harvest and multiple harvests treatment after 2$^{nd}$ cut in 2012 or between no cut (for single harvest) and cut (for multiple harvests) treatment in 2013. Although little is known about how harvest (haying, mowing, or cutting) of biofuel crops affects arthropods, the negative impacts of mowing and increasing mowing frequency on bees, butterflies, and plants are often reported in agricultural systems [32-38]. Mowing shortens vegetation height and removes flowers. This could diminish availability of host plants and nectar sources and lower abundance and richness of beetles (Coleoptera) and hoverflies (Syrphidae) that are sensitive to vegetation height [39]. Thus, the finding of our study is somewhat unexpected. The unusual timing of initial harvest (early April as dormant season harvest) or the summer harvest may influence our results. While initial harvest between late-spring and mid-summer is a common practice to produce good quality hay, delaying the initial harvest until late-summer to fall is recommended for the management of wildlife conservation. The late-growing season cut can reduce the probability of disturbing breeding activities of birds, destroying eggs, and removing food sources for larvae and late-flying insects although its effect would be taxa- and species-specific [34, 40-44]. However, it is unlikely that the timing of harvest strongly affects our results given that we did not find significant effects between no harvest treatment and harvest treatment even after one summer harvest (late-June to early-July) in 2013. Our results could be influenced by the period of post-harvest sampling. We set up traps 2-3 weeks after summer harvest to avoid the bowls being too conspicuous in the short vegetation. But, the vegetation or soil conditions may still provide resources for insects, dissipating the effects of harvest. It is also possible that insects captured in our study, especially, dominant insects (e.g., Dolichopodidae) are less sensitive to changes in vegetation height or other disturbances caused by harvest considering they are abundant and common throughout our study regions. However, these possibilities remain speculative without further study designed with vegetation surveys and more intensive samplings after harvest.

Among two foraging guilds, predators/parasites/parasitoids that play a major role in pest control were prevalent during 3 years of study. One of concerns related to establishment of biofuel crops is potential damages from arthropod pests (e.g., herbivorous insects) [22]. Higher abundance of predators/parasites/parasitoids throughout all years suggests that yield losses by pest problems would be minimal in biofuel production systems tested in our study. On the other hand, we observed decreases of pollinators, especially, substantial reduction (>83% decline in mean abundance per sampling station compared to 2012) in 2013. We do not know potential causes of the decline, however, this drastic decline indicates that additional management could be required to improve the quality of switchgrass and NWSG mix fields for pollinators after the establishment of those biofuel crops.

In our study, abundance of insects varied among fields and sampling stations. While most variations were insignificant between the same treatments, we found several cases of significant differences in abundance or richness between NWSGs mix single harvest (NativeS) and multiple harvests (NativeM) before 1$^{st}$ cut or 2$^{nd}$ cut occurred. This may be partially influenced by vegetation condition of adjacent fields, i.e., matrix quality. Among four NativeS fields, one field showed lower insect abundance than other three fields although insect abundance varied between sampling stations within the field and a year. A half of the field edge was surrounded by two switchgrass fields excluded from analyses due to poor establishment and the other half by non-experimental row crop (soybean or corn) fields. The relatively low quality of matrix and the isolation from other biofuel crops seem to have a negative impact on insect community within the field. This also indirectly supports the importance of considering the landscape context of sites for biodiversity conservation in biofuel cropping systems as well as intensive agriculture [10, 16, 22, 45-47]. We assumed negligible effects of landscape features in our study given the experimental design (e.g., random assignment of treatment within a block) and the narrow geographical range of our experimental fields. However, it is possible that the slight differences in adjacent land covers (e.g., hay or forest) obscure treatment effects by increasing variations in insect responses among fields. We could not incorporate the landscape aspects into our analysis due to small sample size and thus it is unclear what degree landscape features influenced our results if there were.

Another caveat that could affect our results is the insect sampling method, multi-color pan (or bowl) trap. The method is known to be successful at catching a variety of floral visiting insects [48-49] and used to capture other insects. However, it is not efficient to capture surface and ground-dwelling arthropods. In other studies, arthropods are often sampled by more than one method such as pan trap and sweep netting, Malaise trap, or pitfall trap [50-54]. Many surface and ground-dwelling arthropods play a crucial role in ecological processes such as nutrient cycling and in ecosystems by acting as decomposers, predators, and root herbivores [55-58]. Switchgrass is known to form tall and dense vegetation [20, 59]. Thus, microclimate near ground can be significantly different between two biofuel crop types. In addition, harvest can drastically change the microclimate by removing vegetation. Surface and ground-dwelling arthropods may noticeably respond to both harvest and biofuel crop types. We emphasize need of future research to test this possibility by employing multi trapping methods.

## Acknowledgements

We are grateful to B. Bryan Farms for allowing us to conduct this research on their property. We thank T. J. Conkling, M. Thornton, and numerous technicians for data collection. We also thank the Mississippi Agriculture and Forest Experimental Station, the University of Georgia, and the High Point University for their support. The majority of this work was funded by NRCS CIG # 69-3A75-10-158.

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Table 1. Summary of F-values (P-values in parenthesis) from a general linear mixed model tested for abundance of beneficial insects. TOTAL, PO, and PPP represent total beneficial insects (sum of PO and PPP), pollinators, and predators/parasites/parasitoids, respectively. Significant treatment effects (P<0.05) are bolded.

| Year | Response variable | Month | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | May[1] | Jun | Jul[2] | Aug[1,3] | Sep[1,4] | Oct[4] | Nov[1] |
| 2011 | TOTAL | 0.703 | **6.549** | 3.086 | 0.665 | 0.983 | 3.113 | **5.865** |
| | | (0.571) | **(0.010)** | (0.077) | (0.593) | (0.439) | (0.075) | **(0.014)** |
| | PO | 1.355 | **5.167** | 2.236 | 2.293 | 3.508 | 1.699 | **13.155** |
| | | (0.312) | **(0.021)** | (0.147) | (0.140) | (0.057) | (0.23) | **(0.000)** |
| | PPP | 0.080 | **9.220** | **5.092** | 0.256 | 2.516 | 2.912 | 0.296 |
| | | (0.970) | **(0.003)** | **(0.022)** | (0.855) | (0.118) | (0.087) | (0.828) |
| 2012 | TOTAL | 2.915 | 1.900 | 2.506 | 1.988 | 0.851 | **7.076** | |
| | | (0.087) | (0.194) | (0.119) | (0.180) | (0.504) | **(0.008)** | |
| | PO | 0.909 | 0.641 | 2.664 | 2.012 | 1.338 | 0.732 | |
| | | (0.471) | (0.606) | (0.105) | (0.176) | (0.329) | (0.556) | |
| | PPP | 2.940 | 1.486 | 2.259 | 2.308 | 0.788 | **7.039** | |
| | | (0.085) | (0.277) | (0.144) | (0.176) | (0.534) | **(0.008)** | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 2013 | TOTAL | 1.019 | 0.036 | 1.656 | 0.987 | 2.619 |
| | | (0.425) | (0.990) | (0.247) | (0.438) | (0.109) |
| | PO | 0.744 | 1.043 | 2.373 | **9.742** | 2.930 |
| | | (0.550) | (0.415) | (0.132) | **(0.003)** | (0.086) |
| | PPP | 1.092 | 0.054 | 1.387 | 0.601 | 2.429 |
| | | (0.397) | (0.982) | (0.303) | (0.629) | (0.126) |

[1]One trapping occasion in 2011

[2]One trapping occasions at multiple harvests treatment fields (NativeM and SwitchM) in 2013

[3]Three trapping occasions in 2012

[4]One trapping occasion in 2012

Table 2. Summary of F-values (P-values in parenthesis) from a general linear mixed model tested for familial diversity of beneficial insects. S and H represent family richness and Shannon-Wiener diversity index, respectively. Significant or weak treatment effects (P<0.05 or close to P = 0.05) are bolded.

| Year | Response variable | Month | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | May[1] | Jun | Jul[2] | Aug[1,3] | Sep[1,4] | Oct[4] | Nov[1] |
| 2011 | S | 0.241 | 0.124 | 0.639 | 1.630 | 0.512 | **3.686** | 1.484 |
| | | (0.866) | (0.944) | (0.565) | (0.208) | (0.683) | **(0.025)** | (0.243) |
| | H | 1.066 | 0.996 | 0.429 | **4.852** | 1.464 | 1.965 | 1.432 |
| | | (0.406) | (0.411) | (0.734) | **(0.009)** | (0.283) | (0.145) | (0.257) |
| 2012 | S | 0.637 | **5.017** | **3.752** | 2.163 | 0.677 | 0.410 | |
| | | (0.599) | **(0.007)** | **(0.024)** | (0.118) | (0.590) | (0.747) | |
| | H | 1.980 | 0.724 | 0.233 | 0.745 | 0.617 | **8.916** | |
| | | (0.143) | (0.547) | (0.872) | (0.536) | (0.623) | **(0.000)** | |
| 2013 | S | | 0.164 | 0.415 | **3.135** | **8.955** | **3.878** | |
| | | | (0.919) | (0.744) | **(0.043)** | **(0.000)** | **(0.021)** | |
| | H | | **3.411** | 1.564 | **2.926** | 0.938 | 1.573 | |
| | | | **(0.033)** | (0.223) | **(0.053)** | (0.437) | (0.221) | |

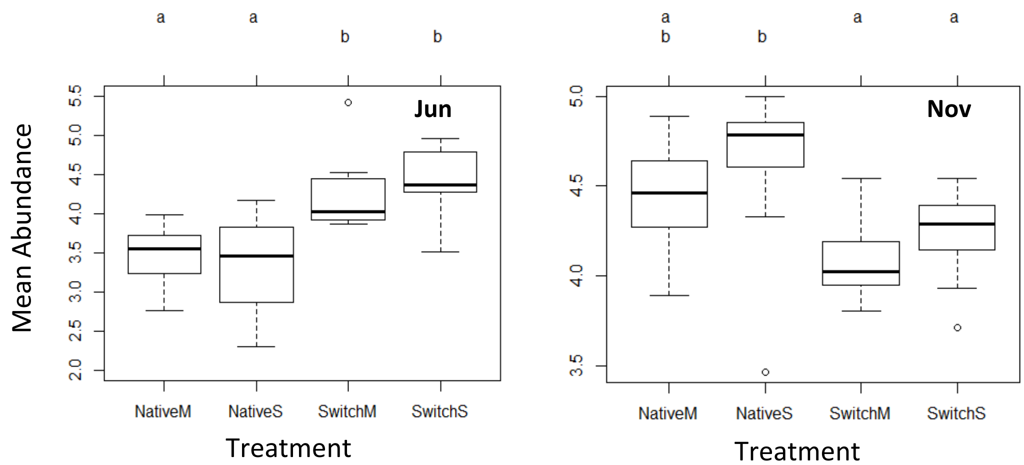
[1]One trapping occasion in 2011

[2]One trapping occasions at multiple harvests treatment fields (NativeM and SwitchM) in 2013

[3]Three trapping occasions in 2012

[4]One trapping occasion in 2012

A. 2011




B. 2012

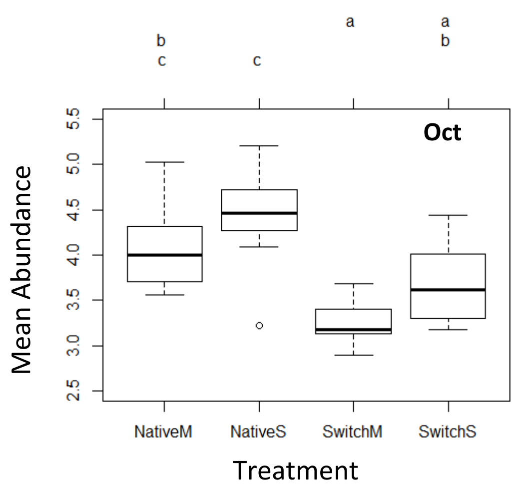



Figure 1. Multiple comparisons (Tukey's HSD test) of total insect abundance between treatments. Treatments with the same letter (a, b, or c) are not significantly different. Areas within a box are 25% quantiles and whiskers indicate the range of the data. The values of abundance are log transformed, log (abundance + 1). Treatment: NativeM, NWSG-mix with multiple biomass harvests; NativeS, NWSG-mix with a single biomass harvest; SwitchM, Switchgrass monoculture with multiple biomass harvests; SwitchS, Switchgrass monoculture with a single biomass harvest.

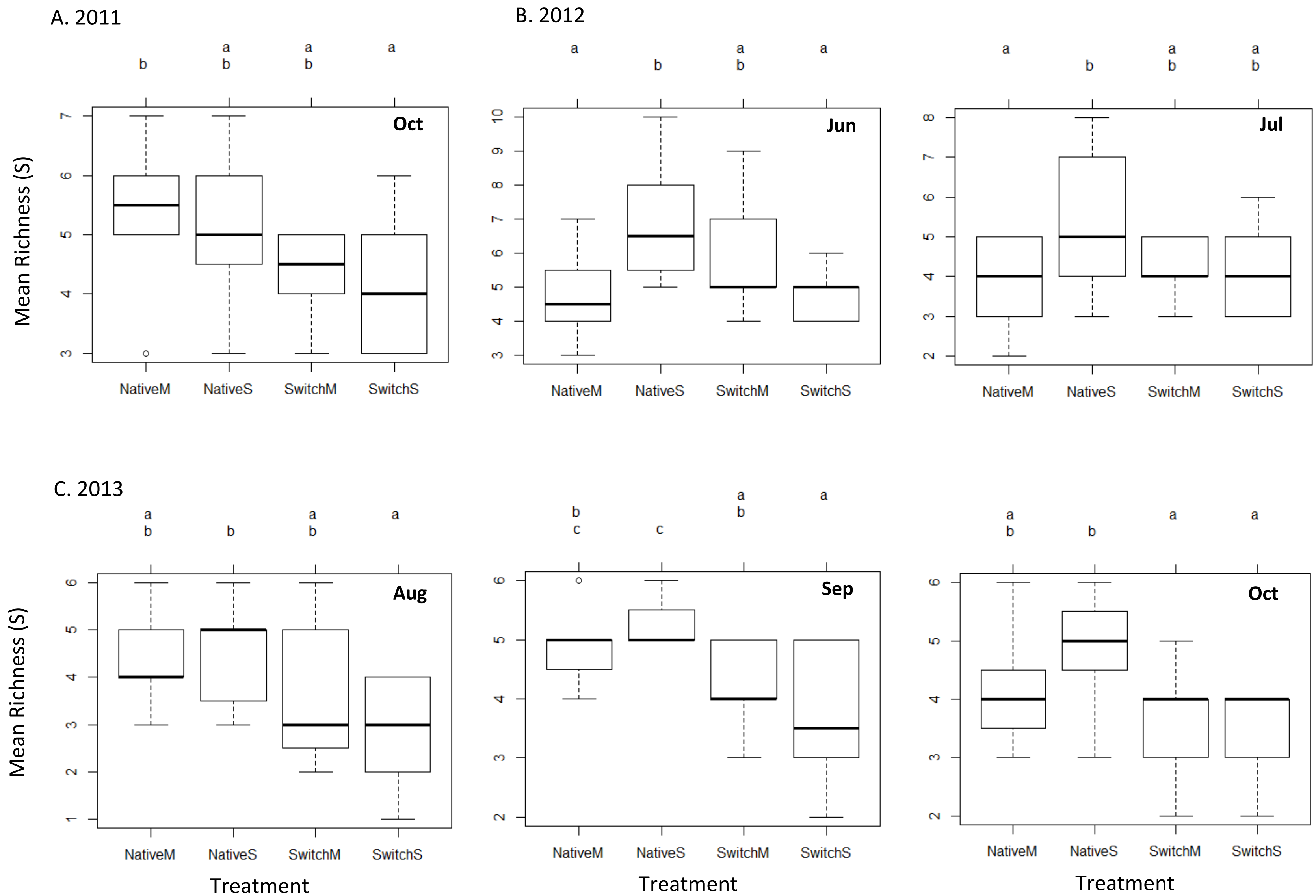
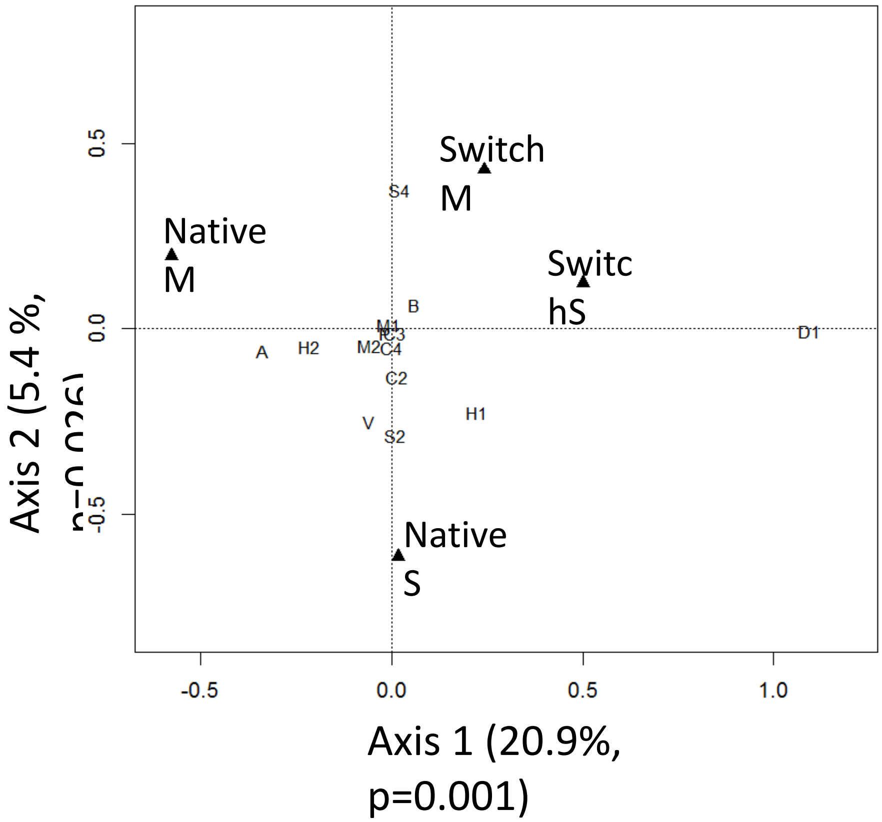
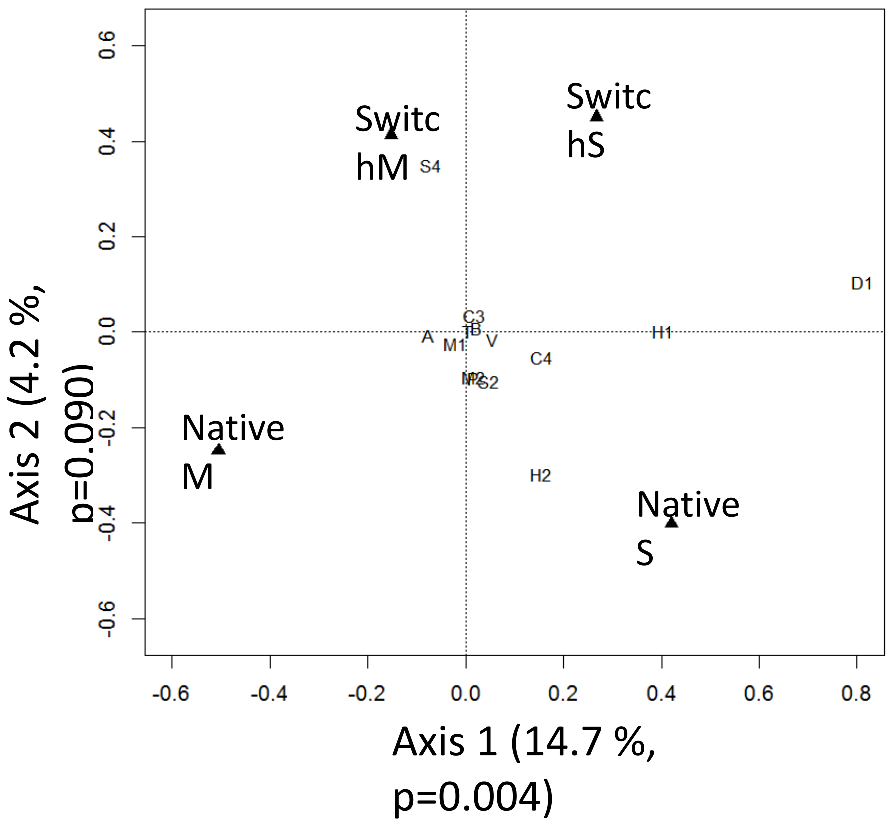

A. 2011
B. 2012
C. 2013
Oct
Jun
Jul
Aug
Sep
Oct
a
b
c
Mean Richness (S)
NativeM
NativeS
SwitchM
SwitchS
Treatment

Figure 2. Multiple comparisons (Tukey's HSD test) of familial richness (S) between treatments. Treatments with the same letter (a or b) are not significantly different. Areas within a box are 25% quantiles and whiskers indicate the range of the data. Treatment: NativeM, NWSG-mix with multiple biomass harvests; NativeS, NWSG-mix with a single biomass harvest; SwitchM, Switchgrass monoculture with multiple biomass harvests; SwitchS, Switchgrass monoculture with a single biomass harvest.

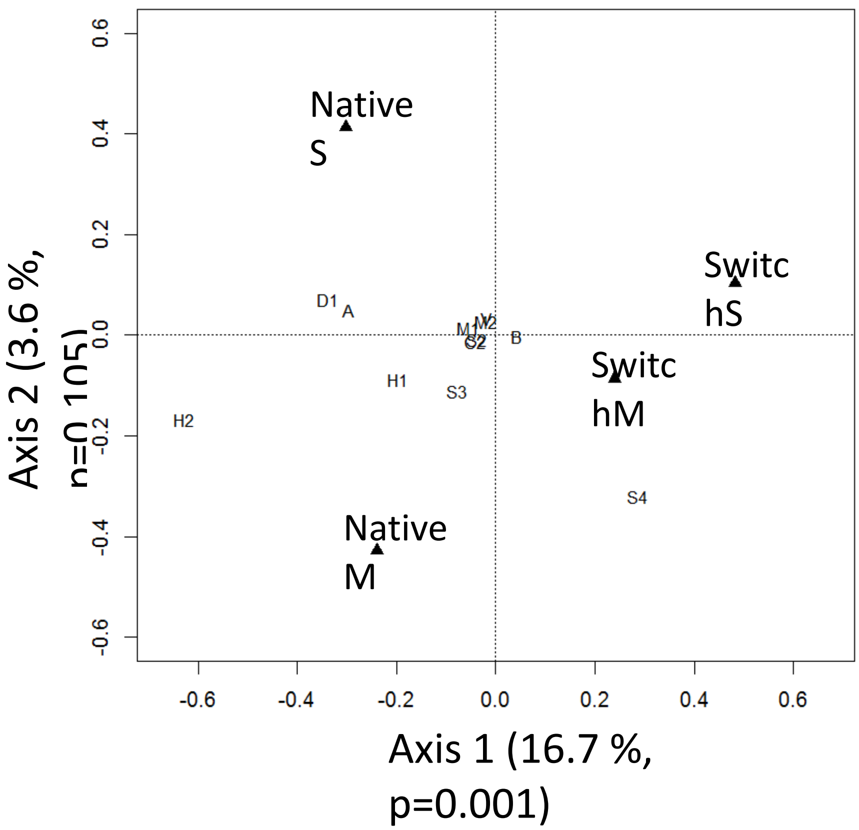
A. 2011


Switch
M
Native
M
Switc
hS
Native
S
S4
B
M1
C3
M2
C4
H2
A
C2
H1
V
S2
D1
0.5
0.0
-0.5
-0.5
0.0
0.5
1.0
Axis 1 (20.9%,
p=0.001)
Axis 2 (5.4 %,

B. 2012


Switc
hM
S4
Switc
hS
D1
C3
H1
A
M1
TB
V
C4
NP2
S2
Native
M
H2
Native
S
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6
0.8
Axis 2 (4.2 %, p=0.090)
Axis 1 (14.7 %, p=0.004)

C. 2013


Native
S
Switc
hS
Switc
hM
Native
M
D1
A
M1
M2
S2
B
H1
S3
H2
S4
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
Axis 2 (3.6 %, p=0.105)
Axis 1 (16.7 %, p=0.001)

Appendix 1. List of species of forbs mixed with native warm season grasses for NWSG mix treatment.

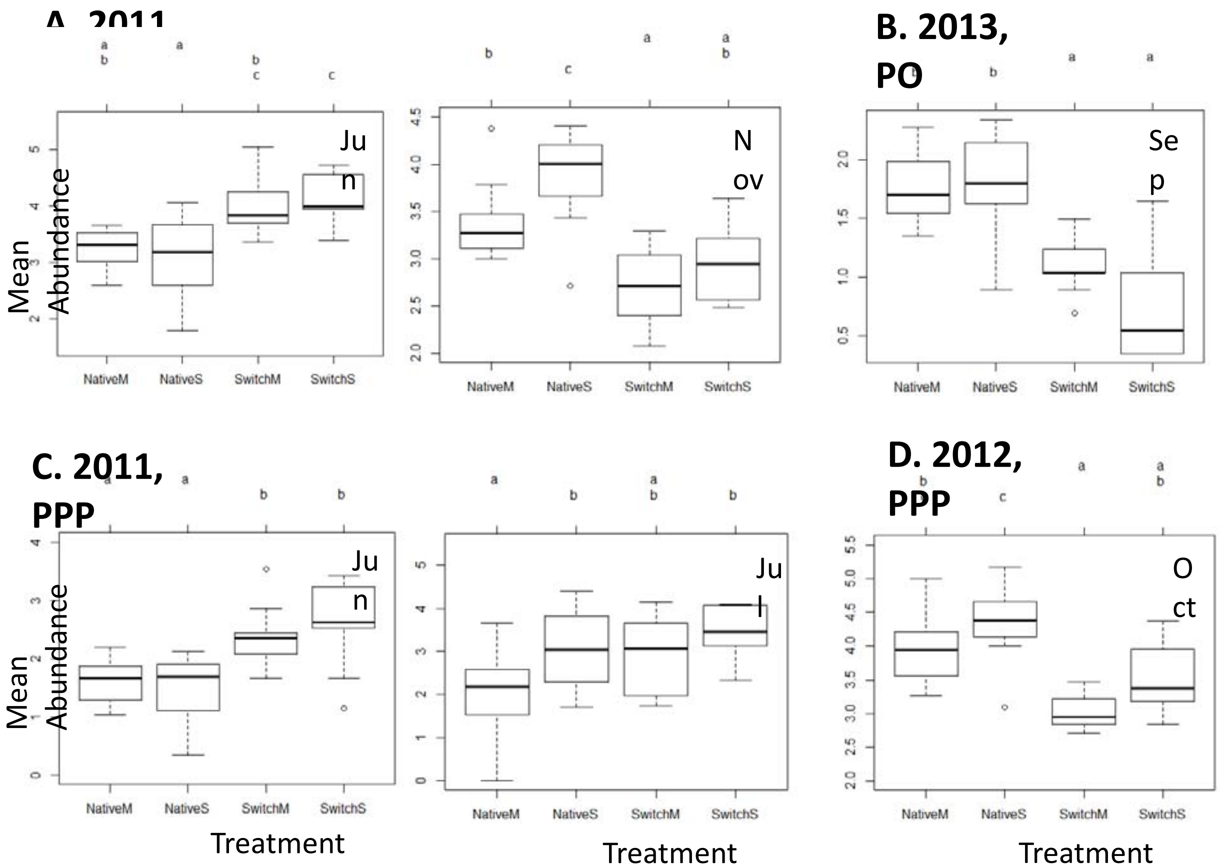
| Common name | Scientific name |
|---|---|
| Big Bluestem | *Andropogon gerardii* |
| Little Bluestem | *Schizachyrium scoparium* |
| Indian Grass | *Sorghastrum nutans* |
| Switchgrass - Alamo | *Panicum virgatum* |
| Roundheaded Lespedeza | *Lespedeza capitata* |
| Greyheaded Coneflower | *Ratibida pinnata* |
| Canada Tick Trefoil | *Desmodium canadensis* |
| Tickseed Sunflower | *Bidens aristosa* |
| Illinois Bundleflower | *Desmanthus illinoensis* |
| Wild Blue Lupine | *Lupinus perennis* |

Appendix 2. Order, family, and guild classification (based on foraging strategy) of all insects captured at Bryan Farms, Mississippi, USA, during 2011-2013.

| **Order** | **Family** | **Foraging strategy** | **No. individuals**[1] |
|---|---|---|---|
| Coleoptera | Buprestidae (B) | Pollinators | 80 |
| | Meloidae (M2) | Predators/parasites/parasitoids | 112 |
| Diptera | Bombyliidae | Pollinators | 7 |
| | Conopidae (C3) | Predators/parasites/parasitoids | 32 |
| | Dolichopodidae (D1) | Predators/parasites/parasitoids | 69404 |
| | Syrphidae (S4) | Predators/parasites/parasitoids | 4996 |
| Hymenoptera (Bees) | Andrenidae | Pollinators | 5 |
| | Apidae (A) | Pollinators | 2031 |
| | Colletidae (C2) | Pollinators | 36 |
| | Halictidae (H1) | Pollinators | 20766 |
| | Megachilidae (M1) | Pollinators | 42 |
| Hymenoptera (Non-bees, Wasps) | Chrysididae | Predators/parasites/parasitoids | 9 |
| | Crabronidae (C4) | Predators/parasites/parasitoids | 58 |
| | Scoliidae | Pollinators | 4 |
| | Sphecidae (S2) | Predators/parasites/parasitoids | 104 |
| | Tiphiidae (T) | Pollinators | 8 |
| | Vespidae (V) | Predators/parasites/parasitoids | 209 |
| Lepidoptera | Hesperiidae (H2) | Pollinators | 707 |
| | Lycaenidae | Pollinators | 5 |
| | Nymphalidae | Pollinators | 1 |
| | Papilionidae | Pollinators | 11 |
| | Pieridae (P) | Pollinators | 37 |
| | Sphingidae | Pollinators | 1 |

[1] Sum of individuals trapped across all sampling occasions during 2011-2013

Appendix 3. Multiple comparisons (Tukey's HSD test) of abundance of insects (pollinators, PO and predators/parasites/parasitoids, PPP) between treatments during 2011-2013. Only months that overall treatment effect was significant are shown (See Table 1 for overall treatment effect). Treatments with the same letter (a, b, or c) are not significantly different. Areas within a box are 25% quantiles and whiskers indicate the range of the data. The values of abundance are log transformed, log(abundance + 1). Treatment: NativeM, NWSG mix with multiple biomass harvests; NativeS, NWSG mix with a single biomass harvest; SwitchM, Switchgrass monoculture with multiple biomass harvests; SwitchS, Switchgrass monoculture with a single biomass harvest.



Appendix 4. Multiple comparisons (Tukey's HSD test) of Shannon-Wiener diversity index (Shannon diversity, H) between treatments during 2011-2013. Only months that overall treatment effect was significant are shown (See Table 2 for overall treatment effect). Treatments with the same letter (a, b, or c) are not significantly different. Areas within a box are 25% quantiles and whiskers indicate the range of the data. Treatment: NativeM, NWSG mix with multiple biomass harvests; NativeS, NWSG mix with a single biomass harvest; SwitchM, Switchgrass monoculture with multiple biomass harvests; SwitchS, Switchgrass monoculture with a single biomass harvest.

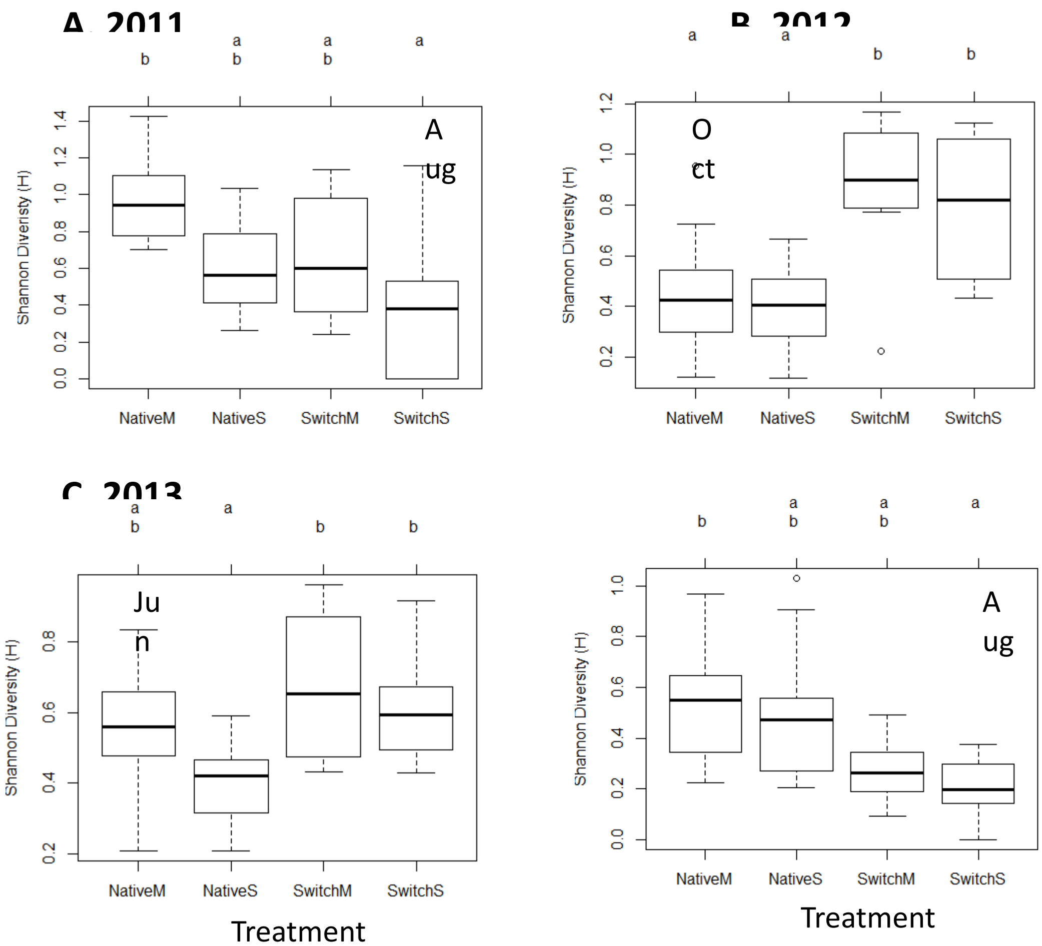


Figure 3. Redundancy analysis (RDA) biplot of four treatments and insect families during 2011-2013. Variance (%) explained by each of the first two axis and *p* values calculated from permutation test of 999 iterations are shown in parenthesis. Treatment: NativeM, NWSG-mix with multiple biomass harvests; NativeS, NWSG-mix with a single biomass harvest; SwitchM, Switchgrass monoculture with multiple biomass harvests; SwitchS, Switchgrass monoculture with a single biomass harvest. Family: A, Apidae; B, Buprestidae; C2, Colletidae; C3, Conopidae; C4, Cicadellidae; D1, Dolichopodidae; H1, Halictidae; H2, Hesperiidae; M1, Megachilidae; M2, Meloidae; P, Pompilidae; S2, Syrphidae; S4, Syrphidae; V, Vespidae; T, Tabanidae.

Appendix H. Insect community response to switchgrass intercropping and timber stand age

# Insect community response to switchgrass intercropping and timber stand age

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Abstract

With growing demand for biofuel feedstocks that can reduce economic and environmental conflicts, industrial pine (*Pinus* spp) landscapes have increasingly received attention as potential resources to produce biofuel feedstocks. Intercropping switchgrass (*Panicum virgatum*) between rows of pine tree in plantations is an emerging method for biofuel feedstock production in forestry systems. As switchgrass intercropping is expected to change vegetation characteristics within a stand, it can influence animal communities in pine plantations, but its effect likely varies with stand age which often determines canopy closure. Therefore, we examined how switchgrass intercropping and stand age (3- to 4-year old, young pine [YPI], and ~10-year old, old pine [OPI]) influenced insect abundance and diversity in loblolly (*P. taeda*) pine plantations in Mississippi, USA during May-August, 2013-2014. We captured insects at 36 locations throughout 12 plots (3 replicates per each of 4 treatments; intercropping and non-intercropping treatment at YPI and OPI stand), using pan traps. Abundance and family-level richness was significantly greater at YPI stand, whereas Shannon-Wiener diversity and evenness at family-level tended to be higher at OPI stand. Conversely, no significant differences were found in insect responses, particularly abundance, between intercropping and non-intercropping treatments. Guild structure and community composition was also strongly influenced by stand age rather than switchgrass intercropping. Our findings suggest that switchgrass intercropping is unlikely to have significant effects on arthropod communities in pine plantations whereas stand age was a main factor affecting arthropods, as often observed in other animal taxa in pine plantations.

Keywords: biofuel feedstock, insects, intensive forestry, Mississippi, *Panicum virgatum, Pinus taeda*

1. Introduction

Pine (*Pinus* spp.) plantations are prevalent throughout the Southeastern USA, comprising approximately 19% of southern forests [1-2]. Although these stands are intensively managed (e.g., site preparation, planting of seedlings, short rotations, fertilization, vegetation control) for commercial timber production, they are also managed with considerations of biodiversity conservation and provide habitat conditions for a variety of animal species, including species of conservation concern [1, 3-5]. However, with growing interest in using these stands to help support a bio-based economy, pine plantations could experience changes in management regimes and thus influence animal and plant community within them [6].

In the USA, there has been intensive research on lignocellulosic biofuel (second-generation biofuel) feedstocks produced from inedible crops or parts of plants as alternatives to annual biofuel crops, especially corn (*Zea mays*) [7-9]. Switchgrass (*Panicum virgatum*), a perennial grass native to the tall grass prairie of the USA, has received great attention as a potential biofuel crop due to its high biomass yield potential, broad adaptability to a wide range of environmental conditions, ease of establishment, and rapid growth [10-14]. Although current biofuel feedstocks are largely concentrated in agricultural systems, intensively managed forest landscapes also contain potential source of lignocellulosic materials that may constitute a marketable biofuel feedstock production system [8, 15-16]. Moreover, herbaceous biomass crops such as switchgrass may be intercropped within pine plantations similar to agroforestry. In this system, switchgrass is planted between rows of pine trees and harvested semi-annually or annually until pine trees shade out switchgrass [17]. In the southeastern USA, switchgrass intercropping has been considered as one potential management regime to produce biofuel feedstocks in forestry systems.

Switchgrass intercropping in pine plantations can provide additional advantages compared to biofuel feedstocks in agricultural systems [18]. Switchgrass intercropping may minimize ecological and economic issues associated with carbon balancing and land conversion, but maximize yields and economic benefits because trees can be harvested as forest products [18]. Switchgrass intercropping could also influence animal diversity in pine plantations because intercropping is likely to alter understory vegetation composition and structure [19]. Switchgrass may be able to create grassland-like habitat conditions suitable for some species [17]. Pine plantations intercropped with switchgrass are also structurally similar to pine-grassland ecosystems that historically flourished across the southeastern USA [6]. A number of studies have investigated or reviewed effects of switchgrass and native warm season grass (NWSGs) plantings on diversity and/or abundance of arthropods, birds, and plants in agricultural biofuel feedstock production systems [20-24]. However, few studies have explored these effects in forest systems. Additionally, findings of those few studies are also variable. For example, responses of birds to switchgrass intercropping were different based on their ecological guild and year [25] but rodents displayed low evenness and diversity within pine plantations intercropped with switchgrass [26], and diversity and abundance of herpetofauna were not influenced by switchgrass intercropping [27]. Although these studies have provided some insight on relationships between switchgrass intercropping and animal diversity in feedstock production systems in forests, most of them have centered on small vertebrates. Responses of arthropods, which are key providers of ecosystem services [28], to switchgrass intercropping have rarely been examined.

Therefore, to better understand potential effects of intercropping switchgrass on biodiversity, we investigated effects of switchgrass intercropping on abundance, diversity at family-level, and foraging guilds of insects in loblolly pine plantations. Unlike previous studies (see Loman et al. 2014[25] for exception) centered on specific stand age (e.g., 2-, 5-, or 7-year old stand), we considered two stand age classes: an approximately 10-year old pine stand (hereafter, OPI stand) with a closing pine canopy and a less than 5-year old stand (hereafter, YPI stand) with an open pine canopy. In pine stands, amount of canopy cover (canopy closure or openness) positively associated with stand age is one of major factors affecting vegetation structure and heterogeneity and biodiversity within stands [29-30]. Pine stands often show diverse understory vegetation and increased wildlife use during early years when the canopy is open. However, this diversity and use decreases as the canopy closes [5, 31-32]. Thus, influence of switchgrass intercropping on insect communities likely varies depending on stand age. We hypothesized that although abundance and diversity of insects could be greater in YPI stands than OPI stands, switchgrass intercropping would have more positive effects on the insect community in OPI stand via increased herbaceous understory vegetation as compared to YPI stands.

## 2. Materials and methods

### 2.1. Study site and treatment

Our study was conducted on land owned and managed by Weyerhaeuser Company in Kemper County, Mississippi, USA between late spring and summer during 2013-2014. Approximately 70% of this landscape was composed of loblolly pine (*Pinus taeda*) plantations with the remainder being mixed pine-hardwood or hardwood forests and forest openings. Our study plots were established and maintained by Catchlight Energy LLC, a joint venture between Chevron and Weyerhaeuser Company, and Weyerhaeuser Company.

During 2013, we established 2 plots each, 9-10 ha in size, within 3, 3-4 years old, loblolly pine stands (YPI stands). Trees were planted in a 1.5m x 6.1m arrangement during winter 2010-2011 after clearcutting existing pine stands in 2009-2010. One plot was a control (pine stand without intercropped switchgrass; PI) that followed standard Weyerhaeuser silvicultural practices and the other was pine intercropped with switchgrass (SI). Switchgrass was seeded and re-seeded during May-June in 2011and 2012, respectively and harvested during fall and winter, 2013. In addition to these stands, we also chose six ~ 9-10 years old loblolly pine stands (OPI stands) planted in 2004 and established one plot (9-10 ha) in each stand: three plots received switchgrass intercropped treatment (T-PISI) and the other three plots no intercropping treatment (T-PI). Thus, our experiment included 12 plots (3 replicates per each of 4 treatments; intercropping and non-intercropping treatment at YPI and OPI stand. Switchgrass in T-PISI was seeded in 2009 and annually harvested in fall or winter. Woody debris and stumps in all plots (SI and T-PISI) with intercropped switchgrass were removed between pine tree rows using a bulldozer with a V-blade plow (See Loman et al. 2014 for the detail description of all stands and plots).

2.2. Insect collection

We prepared 3 different colored (blue, white, and yellow colored), 12 oz plastic bowls as one set of pan traps for insect sampling. We filled bowls with a soap-water solution as a trap medium. Within each plot, we established 3 sampling stations located at least 50 m from stand edges to avoid edge effects and ≥ 50 m from nearest other sampling location to minimize dependency among pan traps. In the case of SI and T-PISI, we located a sampling station within a switchgrass row but closer to pine beds than center of switchgrass rows (switchgrass was planted in a 3 m swath within 4.9 m interbed rows). We placed one set of bowls to height of flowers or vegetation at each sampling station. We established 36 sampling stations (3 stations x 12 plots) across study sites.

We trapped insects during May - August in 2013 and 2014. We installed one set of bowl traps at each sampling station once a month in May and twice (about 10-14 days apart) a month for the other periods except August (three trapping occasions) in 2014. We collected contents of each trap three days following trap set up and preserved insect samples in a 70% ethanol solution for identification.

2.3. Data analyses

We counted and identified all insects to family level with an expert (Joshua W. Campbell, Highpoint University). We grouped insects into 4 guilds according to their foraging strategy (Table 1): predators/parasites/parasitoids (PPP), pollinators (PO), herbivores (HE), and "others", the latter which included insect families that could not be clearly classified into one of three foraging guilds (PPP, PO, HE) largely because of variable foraging strategy depending on genus. Among families used for analysis, we only classified one family (Mordellidae) as "others."

To examine effect of treatment on insect abundance and insect diversity at family level, we used abundance (number of individuals) of all insects (Total), abundance of guild, richness (S, number of families), Shannon-Wiener diversity index (Shannon diversity, H) and Pielou's evenness index (Evenness, E) as response variables. For abundance of guild, we focused on three guilds (PPP, PO and HE) due to their importance in providing ecosystem services such as pest control (PPP), pollination (PO) and potential pest problems (HE). We conducted a separate analysis for each month and used mean value of each metric per sampling station as response during each month. We log(x+1)-transformed abundance prior to analyses to avoid biases due to too high abundance of some families at sampling stations. For response variables (S, H and E) associated with diversity index, we excluded families detected at <4 sampling stations (<10% of total number of sampling stations) to avoid biases due to occurrence of uncommon families. We used 19 and 20 families for these analyses in 2013 and 2014, respectively.

We examined homogeneity of variance assumption using Levene's test. When violation of this assumption was found, we allowed different variances among treatments in the model ([33]. To test significance of treatment effects, we used a general linear mixed model with treatment as a fixed effect and plot as a random effect because we considered a sampling station as the experimental unit and sampling stations were nested within plots. We also conducted Tukey's HSD post hoc test for multiple pair comparisons when a significant effect ($P<0.05$) was found. All of these analyses were performed in R, using package "nlme" [34] and "multcomp"[35] for overall treatment effect test and Tukey's HSD post hoc test, respectively. We also calculated diversity metrics (S, H, and E) in R with package "vegan" [36].

We investigated relationships between treatment and structure of insect community using analysis of similarity (ANOSIM) and redundancy analysis (RDA). Analysis of similarity tests whether there is a significant compositional difference between groups by comparing distances between groups with distances within groups. We used Bray-Curtis similarity as a measure of distance. Values of ANOSIM statistic R range from 1 to -1: R value from 0 to +1 indicates increasing dissimilarity among groups; R value=0 indicates no relationship; and R value from 0 to -1 indicates similarity among groups (more than within a group). RDA is a canonical ordination method commonly used to analyze community composition data. It is a combination of regression analysis and principal component analysis [37]. We pooled all monthly data and performed analysis by year. We used mean log(x+1)-transformed abundance of each family captured at each sampling station (i.e., bowl set-up). We conducted ANOSIM and RDA analyses in R with package “vegan”. In addition to ANOSIM and RDA, we also calculated relative frequency (or proportion) of 4 foraging strategy guilds, based on mean abundance of each guild at each treatment, to explore variations in guild structure among treatments.

## 3. Results

### 3.1. General pattern

We trapped 15,571 individual insects of 29 families during 2013-2014 (Appendix A). Although there was one more trapping occasion in 2014, number of insects captured was higher in 2013: 8,604 in 2013 and 6,967 in 2014. We captured insects more frequently during June both years. Among 29 families, Dolichopodidae and Cicadellidae were the most abundant, comprising 61.3% (2013) and 57.8% (2014) of insects captured. We captured 10 and 9 families at < 4 sampling stations in 2013 and 2014, respectively (Appendix A).

### 3.2. Effect of treatment

*Abundance*

Abundance of TOTAL and PPP was significantly influenced by treatments both years (Table 2). Significant differences were largely found between YPI stand (PI and SI) and OPI stand (T-PI and T-PISI), with higher abundance at YPI stand (Fig. 1 and Appendix B and C). While herbivores (HE) also showed similar patterns, significant treatment effect was more often observed between PI and T-PI (pine stands without switchgrass intercropping but different age), particularly in 2014 (Appendix C). Pollinators (PO) did not show significant responses to treatments except August 2014 (Table 2 and Appendix C). Unlike the strong effect of stand age on insect abundance, switchgrass intercropping did not show significant influence given that mean abundance was similar within same aged-stands except one month. During August 2014, TOTAL and PPP was significantly low at T-PISI than at T-PI.

*Family-level diversity*

Treatment showed significant or weak effect on diversity metrics (family richness, Shannon diversity, evenness) in some comparisons (Table 2). Similar to insect abundance, richness (S) at YPI stand was significantly greater than at OPI stand during July, 2013 and during July-August, 2014 (Fig. 2). In 2013, of 20 families trapped at >4 sampling stations, only 12 families were observed at T-PI throughout 4 months, whereas 18-19 families were found at other three treatments. In 2014, YPI stand and OPI stand had 19 and 15 families, respectively. Conversely, Shannon diversity (H) and evenness (E) tended to be higher at OPI stand in 2013 (Fig. 2 and Appendix D). We found significant effect of switchgrass intercropping only in 2013, between T-PI and T-PISI (higher richness at T-PISI) during June and between PI and SI (higher Shannon diversity at SI) during May (Fig. 2).

*Composition of insect community*

Insect community structure based on foraging strategy guild was also more affected by stand age than intercropping treatment. In 2013, insect community at YPI stand was more composed of PPP and HE whereas insect community at OPI stand was dominated by both PPP and PO (Fig 3, A). In 2014, similar pattern was found at OPI stand (Fig. 3, B). Compared to 2013, relative proportion of pollinators increased at all stands, especially at YPI stand in 2014. At the same stand age class, relative proportion of each guild was similar except PI in 2014.

Relative proportion of herbivore was higher at PI than at SI whereas predators/parasites/parasitoids showed an opposite pattern.

Family composition among four treatments was significantly different compared to within a treatment: ANOSIM statistic R = 0.45 (p = 0.001) and 0.45 (p = 0.001) in 2013 and 2014, respectively. Those significant differences were largely influenced by stand age based on RDA results which showed that treatment explained 50.6% (2013) and 59.4% (2014) of variation in our data. In particular, the first canonical axis from RDA (Axis 1) that represented a gradient between YPI stand and OPI stand explained 48.2% (2013) and 55.4% (2014) of variation, suggesting a stronger influence of stand age on insect family composition than switchgrass intercropping (Fig. 4). Although almost half of families analyzed did not show clear associations with treatment, more families were associated with YPI stand than OPI stand. Mordellidae (M4) and Halictidae (H1) were positively related to YPI stand both years. Although Apidae (A2) and Syrphidae (S2) were associated with OPI stand in 2013, they showed more association with YPI stand in 2014. Cicadellidae (C4) and Dolichopodidae (D) were also positively related to YPI stand in 2013.

## 4. Discussion

In agricultural systems, numerous studies have documented greater diversity and abundance of birds, arthropods, and plants in switchgrass stands over corn [20-24]. On the contrary, in forestry systems where only a few studies have explored responses of animal to switchgrass intercropping, effect of switchgrass intercropping appears to vary depending on taxa, parameter of interest, years since switchgrass establishment, and temporal variation. In Mississippi, USA, intercropping negatively influenced bird abundance at <5-year old stand during the first growing season [25], which was affected by site preparation process for intercropping, but positively influenced bird abundant during the second growing season, although there were some variations in responses among guilds. Intercropping also lowered rodent diversity at 7-years by increasing abundance of a common species, but did not affect survival or recruitment of any species [26]. In North Carolina, USA, there were little differences in rodent community composition [38] and diversity and abundance of amphibian and reptiles [27] between intercropped and non-intercropped treatment in treatment plots less than 5 years old. Intercropping also did not affect diet and trophic position of native rodent species in North Carolina [39].

Similar to the North Carolina studies, in our study, intercropping switchgrass did not have significant effects on insect communities, particularly abundance. Within the same stand age class, we did not find any significant differences in abundance between intercropped treatment and non-intercropped treatment except in one month (August in 2014). Although several families showed weak correlation with SI (Mordellidae) and PI (Halictidae and Syrphidae) in 2014, most families were not associated with intercropping treatment. However, we note that, in 2013, T-PISI and SI showed relatively greater species richness (June) and Shannon-Wiener diversity (May) compared to T-PI and PI, respectively. This may suggest that switchgrass intercropping may increase insect diversity during late spring-early summer although this could change as stands mature.

However, overall insignificant effects of switchgrass intercropping across two stand age classes did not support our hypothesis. In general, >7-year old stands, such as T-PI stands in our study, have a more closed canopy until thinning and thus contain relatively less herbaceous vegetation, whereas ground layer of young pine stand with open canopy such as PI is more often covered by diverse herbaceous vegetation [40]. Thus, switchgrass intercropping may significantly change vegetation composition and structure within OPI stand by increasing grassy and other herbaceous understory vegetation. Given the well-known positive relationship between heterogeneous vegetation and arthropod community [41-43], we expected stronger positive effects of intercropping on insect community at OPI (10-year old) stand than at YPI (<5-year old) stand. Due to lack of vegetation data, it is unclear how stand age and intercropping influenced vegetation characteristics and whether the pattern found in our study was related to those characteristics. However, one recent study showed insignificant differences in plant diversity between intercropping treatment and non-intercropping treatment at young pine stand (similar to YPI) in our research site [44]. Dense shrub/woody vegetation within OPI stands and at edges of switchgrass rows adjacent to pine beds was commonly observed throughout our study sites (Craig Marshall, Mississippi State University, per.comm.). In addition, Loman et al. [25] demonstrated decline in density of pine-grassland birds at intercropped stands when those stands reached 8-year old (the last year of their 3 years of study duration). Considering that pine-grassland birds are habitat specialists, inhabiting open pine forest with herbaceous understory vegetation, these results indicated that vegetation changes associated with intercropping may remarkably decrease as stands matures and pine trees shade intercropped crops. Loman et al. [25] also reported increasing semi-woody vines and shrubs even at <5-year old stand intercropped with switchgrass as stands matured. Based on these patterns, shrub/woody vegetation dominant in pine stands with closed canopy seems to outcompete herbaceous vegetation created by switchgrass intercropping and diminish potential positive effects of intercropping. A similar successional pattern has been well-documented in non-intercropped pine stands [40].

Unlike switchgrass intercropping, pine stand age strongly affected insect abundance, diversity, and composition in pine plantations. Overall, we found significantly greater insect abundance and richness, but lower Shannon-Wiener diversity and evenness at YPI stand than at OPI stand. More families were also associated with YPI stand. Stand age is one major consideration for wildlife management in pine forests because it influences level of canopy closure that determines vegetation structure within a stand [29]. Therefore, significant effect of stand age was expected and congruent with patterns (low abundance and diversity of, e.g., birds) frequently observed at >7-year old pine stands until thinning compared to early successional pine stands <5-year old stand [30, 43]. Although low Shannon-Wiener diversity and evenness at YPI stand may be seen as contradictory, this is due to close association of frequently captured insect families (Cicadellidae, Dolichopodidae, Halictidae, and Mordellidae) within YPI stand. Those families were 3-40 times more abundant than other families at YPI stand, whereas 1.5-25 times at OPI stand. This skewed distribution of abundance among families lowered values of those diversity indices despite greater richness at YPI stand.

Among guilds analyzed in our study, PPP and PO are often considered as beneficial insects due to ecosystem services (pest control and pollination) they can provide, whereas HE are potential pest insects that can damage crops [45-50]. We found higher relative proportion of herbivores within an insect community at YPI stand than at OPI stand in 2013. However, during 2014, relative proportion of herbivores decreased but that of beneficial insects increased at SI. Relatively higher proportion of herbivores at YPI stand is not surprising because herbaceous vegetation cover was likely high within the stand. Variation observed at SI suggests that intercropping at early successional pine stands may enhance ecosystem services once switchgrass is well established.

In conclusion, our study provides another insight on assessing environmental sustainability of switchgrass intercropping regarding arthropod communities (abundance and diversity) in forest-based biofuel feedstock systems. Our findings indicate that switchgrass intercropping in pine plantations is unlikely to have significant impacts on insect communities (diversity, abundance, and composition) probably due to the dominant effect of stand age in pine systems. However, it should be pointed out that there are several caveats to our study. First, Mordellidae includes beetles that can be classified as predators/parasites/parasitoids, pollinators, or herbivores depending on their genus and life stage. Due to difficulties in identifying this family to genus level, we classified Mordellidae as “others”, following Robertson et al [22]. Although Mordellidae was not the most abundant family in our study, it comprised 11% of total insects captured. Thus, it is possible that abundance of guild and guild structure at each treatment would slightly change depending on foraging strategy guild of Mordellidae.

Second, insects sampled in our study may be biased by using pan (or bowl) traps. Pan traps, particularly multi-color pan traps similar to those in our study, successfully capture a wide diversity of floral-visiting insects [51-52]. While pan traps have been used to capture other insects, arthropods are often sampled by more than one trapping method. For example, sweep netting, Malaise traps, or pitfall traps are several other ways that researchers have sampled diverse insect communities [53-57]. More importantly, pan traps, are not the most efficient way to capture surface and ground-dwelling arthropods. Many of those arthropods act as decomposers, predators, or root herbivores and are important for soil structure and nutrient cycling [58-61]. If switchgrass increases density of vegetation on the ground and alters microclimate or amount of organic debris, surface and ground-dwelling arthropods may respond significantly. Although any bias associated with using pan traps should be consistent among treatments and should not affect inferences from our data, we emphasize need of future research to test this possibility by employing different trapping methods.

Future studies also need to consider mixtures of perennial biofuel crops (e.g., NWSGs or NWSGs-forbs mix) as intercropping crops and examine how polycultures affect arthropod communities. In agricultural biofuel feedstock systems, a growing body of literature supports that compared to switchgrass (and other biofuel crop) monoculture, polyculture of herbaceous grassland plants enhances biodiversity [11, 24, 62]. Polycultures also lower nutrient inputs and may increase biomass yield, depending on type and number of species mixed [63]. However, mixed crops reduce efficiency of methods to extract biofuels from feedstocks. Likewise, using mixtures of perennial herbaceous plants as intercropping crops may lead to noticeable changes in arthropod communities and other animal and plant communities within early successional pine stands.

Acknowledgements

We thank Catchlight Energy LLC, a Chevron-Weyerhaeuser joint venture, and Weyerhaeuser Company for support. D. A. Miller provided support under a Statement of Work as a Service Provider for Catchlight Energy LLC during this research.

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Table 1. Order, family, and guild classification (based on foraging strategy) of all insects detected at Scooba site, Mississippi during 2013-2014.

| Order | Family[1] | Foraging strategy |
|---|---|---|
| Coleoptera | Buprestidae (B3)[2] | Pollinators |
| | Cerambycidae (C1) | Herbivores |
| | Meloidae (M2) | Predators/parasites/parasitoids |
| | Mordellidae (M4) | Others/omnivores |
| Diptera | Bombyliidae (B2) | Pollinators |
| | Dolichopodidae (D) | Predators/parasites/parasitoids |
| | Syrphidae (S2) | Predators/parasites/parasitoids |
| | Tabanidae (T) | Pollinators |
| Hemiptera | Alydidae | Herbivores |
| | Coreidae | Herbivores |
| | Reduviidae | Predators/parasites/parasitoids |
| Homoptera | Cercopidae (C2) | Herbivores |
| | Cicadellidae (C4) | Herbivores |
| | Membracidae (M3) | Herbivores |
| Hymenoptera (Bees) | Andrenidae (A1) | Pollinators |
| | Apidae (A2) | Pollinators |
| | Colletidae (C5) | Pollinators |
| | Halictidae (H1) | Pollinators |
| | Megachilidae (M1) | Pollinators |
| Hymenoptera (Non-bees, Wasps) | Chrysididae (C3) | Predators/parasites/parasitoids |
| | Crabronidae (C6) | Predators/parasites/parasitoids |
| | Pompilidae (P) | Predators/parasites/parasitoids |
| | Scoliidae[3] | Pollinators |
| | Sphecidae (S1)[4] | Predators/parasites/parasitoids |
| | Tiphiidae[5] | Pollinators |
| | Vespidae (V) | Predators/parasites/parasitoids |
| Lepidoptera | Hesperiidae (H2) | Pollinators, Herbivores |
| | Sphingidae | Pollinators |
| | Satyridae | Pollinators |

[1] Families included in redundancy analysis are indicated with abbreviation in parenthesis
[2] Insects belonging to B2 also feed on dead trees.
[3] This family can be classified as predators/parasites/parasitoids.
[4] This family can also be pollinators.
[5] This family can also be predators/parasites/parasitoids.

Table 2. Summary of F-values (P-values in parenthesis) from a general linear mixed model with treatment as fixed effect and plot as random effect. Abbreviations: Total, all insects; PPP, predator/parasite/parasitoid; PO, pollinator; HE, herbivore; S, family richness; H, Shannon diversity; E, evenness.

| Response variable | | 2013 | | | | 2014 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | May[1] | June | July | August | May[1] | June[1,2] | July | August |
| Abundance | Total | 10.414 | 9.587 | 9.090 | 6.785 | 20.189 | 12.200 | 9.508 | 20.446 |
| | | (0.004) | (0.005) | (0.006) | (0.014) | (0.000) | (0.002) | (0.005) | (0.000) |
| | PPP | 25.800 | 16.723 | 3.356 | 5.881 | 20.140 | 5.856 | 13.912 | 19.681 |
| | | (0.000) | (0.000) | (0.076) | (0.020) | (0.000) | (0.020) | (0.002) | (0.000) |
| | PO | 0.330 | 0.426 | 1.010 | 0.040 | 1.326 | 2.858 | 1.960 | 8.035 |
| | | (0.804) | (0.740) | (0.437) | (0.989) | (0.332) | (0.105) | (0.120) | (0.009) |
| | HE | 0.287 | 2.385 | 7.112 | 10.056 | 0.743 | 4.650 | 2.943 | 17.637 |
| | | (0.833) | (0.145) | (0.012) | (0.004) | (0.556) | (0.037) | (0.099) | (0.000) |
| Diversity | S | 0.855 | 4.213 | 5.211 | 3.675 | 2.478 | 2.254 | 3.381 | 6.584 |
| | | (0.502) | (0.046) | (0.028) | (0.063) | (0.136) | (0.159) | (0.075) | (0.015) |
| | H | 8.493 | 8.183 | 1.251 | 2.620 | 0.321 | 0.272 | 0.839 | 1.714 |
| | | (0.007) | (0.008) | (0.354) | (0.123) | (0.810) | (0.844) | (0.510) | (0.241) |
| | E | 13.278 | 5.597 | 1.316 | 0.519 | 1.558 | 2.357 | 2.646 | 2.581 |
| | | (0.002) | (0.023) | (0.335) | (0.681) | (0.273) | (0.148) | (0.121) | (0.126) |

[1] One sampling occasion

[2] Three sampling occasions

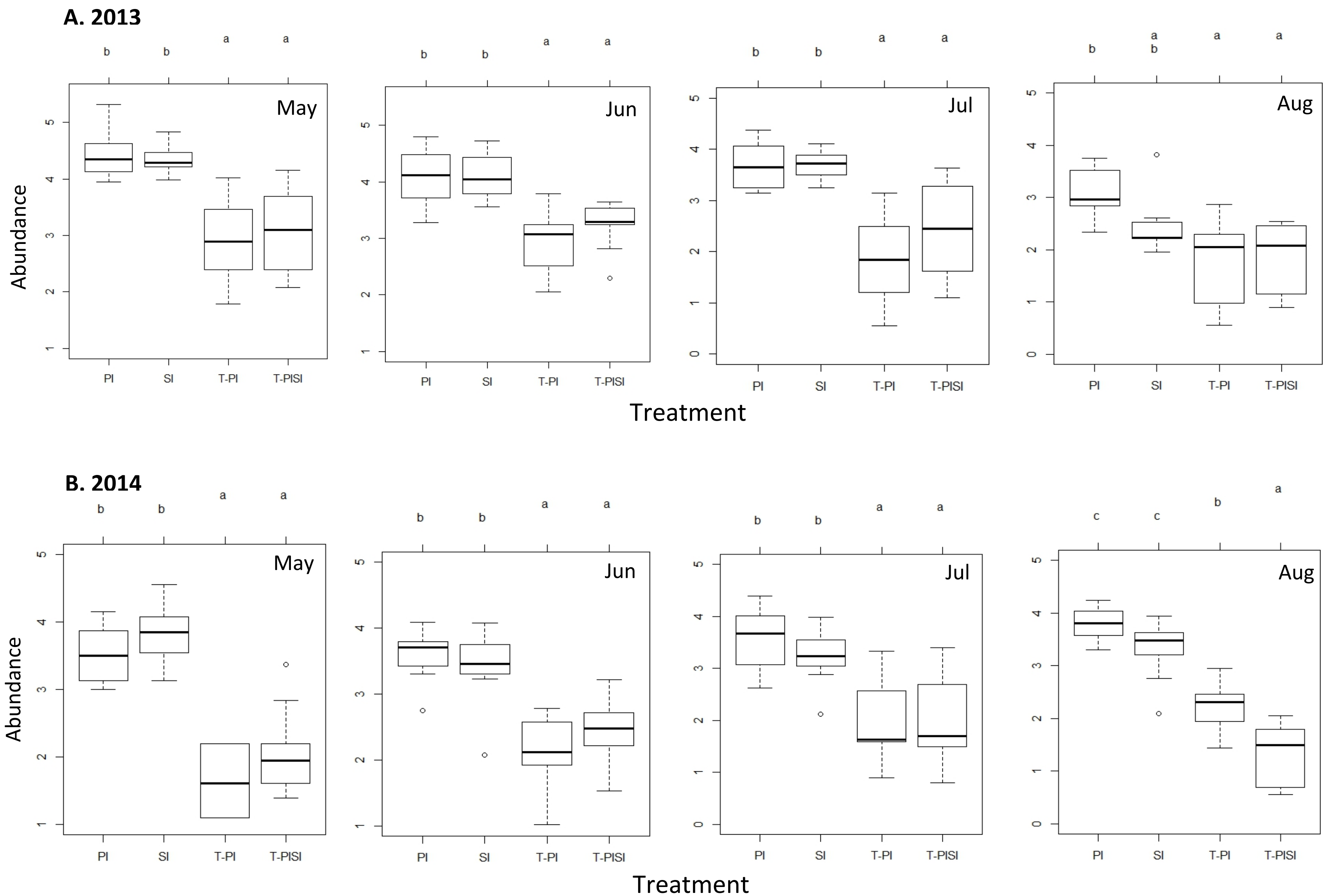
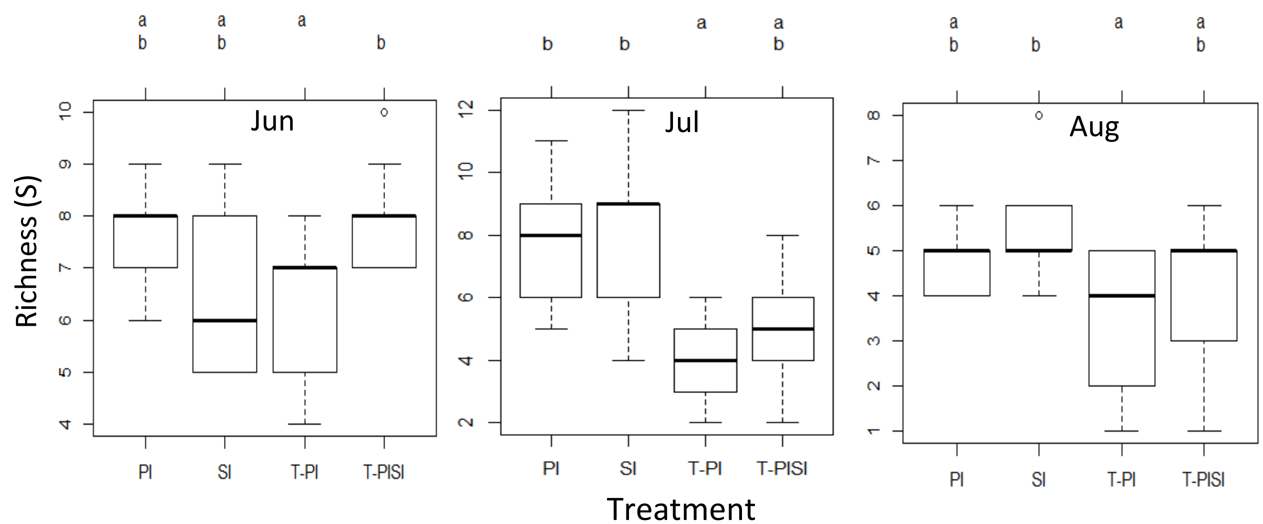
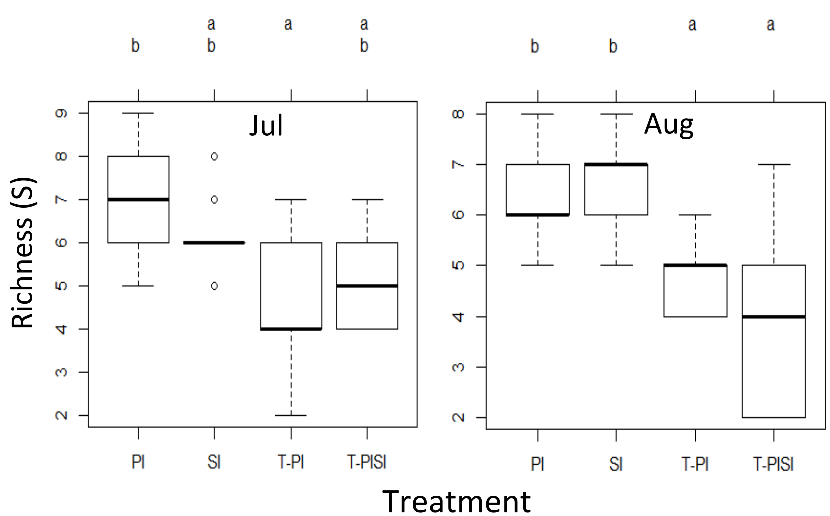

A. 2013
May
Jun
Jul
Aug
Abundance
PI
SI
T-PI
T-PISI
Treatment
B. 2014
May
Jun
Jul
Aug
Abundance
PI
SI
T-PI
T-PISI
Treatment

Fig 1. Multiple comparisons (Tukey's HSD test) of total insect abundance (A and B for year 2013 and 2014, respectively) between treatments. Treatments with the same letter (a, b, or c) are not significantly different. Areas within a box are 25% quantiles and whiskers indicate the range of the data. Treatment: T-PI, OPI stand with no intercropped switchgrass; T-PISI, OPI stand with intercropped switchgrass; PI, YPI stand with no intercropped switchgrass; SI, YPI stand with intercropped switchgrass.

**A. 2013, Richness**




**B. 2014, Richness**




**C. 2013, Shannon diversity**

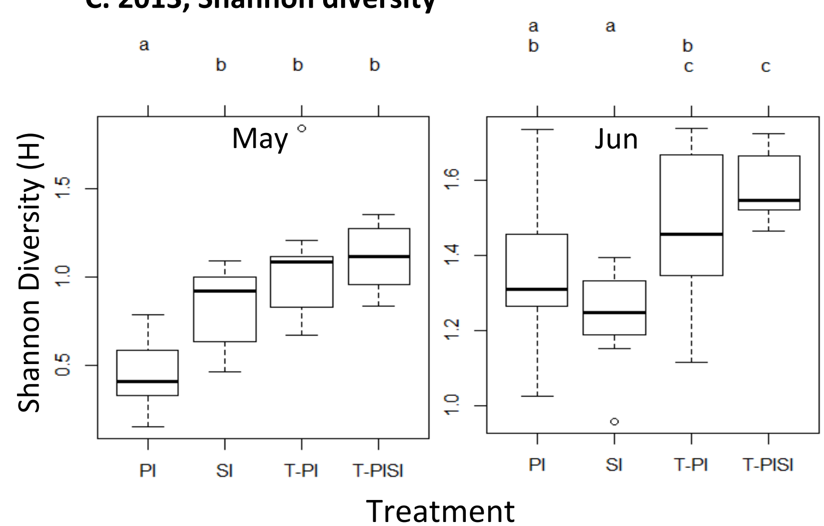


Figure 2. Multiple comparisons (Tukey's HSD test) of family-level richness (A and B) and Shannon diversity (C) between treatments. Only months that overall treatment effect was significant are shown (See Table 2 for overall treatment effect). Note that there was no significant treatment effect on Shannon diversity in 2014. Treatments with the same letter (a, b, or c) are not significantly different. Areas within a box are 25% quantiles and whiskers indicate the range of the data. Treatment: T-PI, OPI stand with no intercropped switchgrass; T-PISI, OPI stand with intercropped switchgrass; PI, YPI stand with no intercropped switchgrass; SI, YPI stand with intercropped switchgrass.

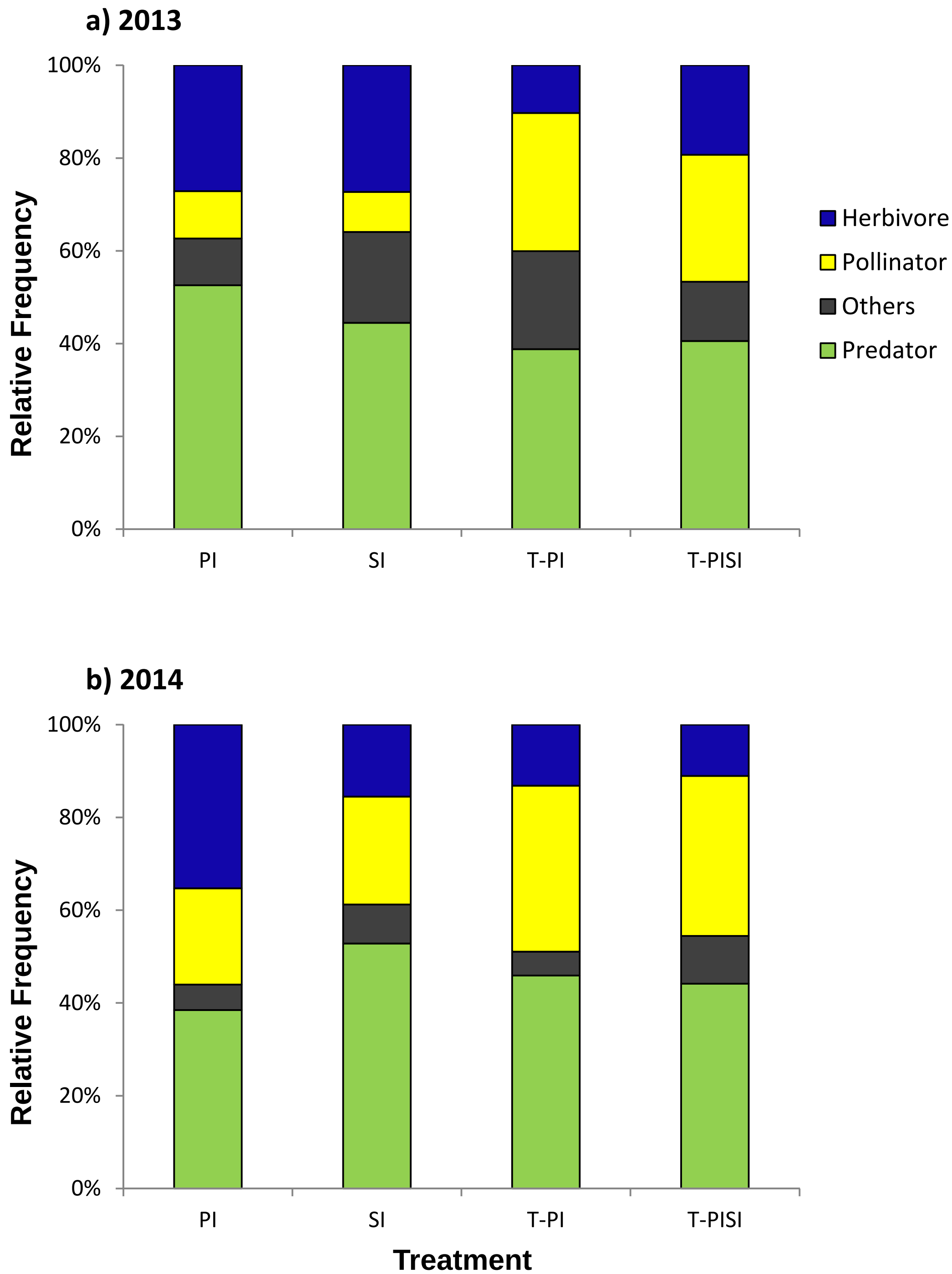
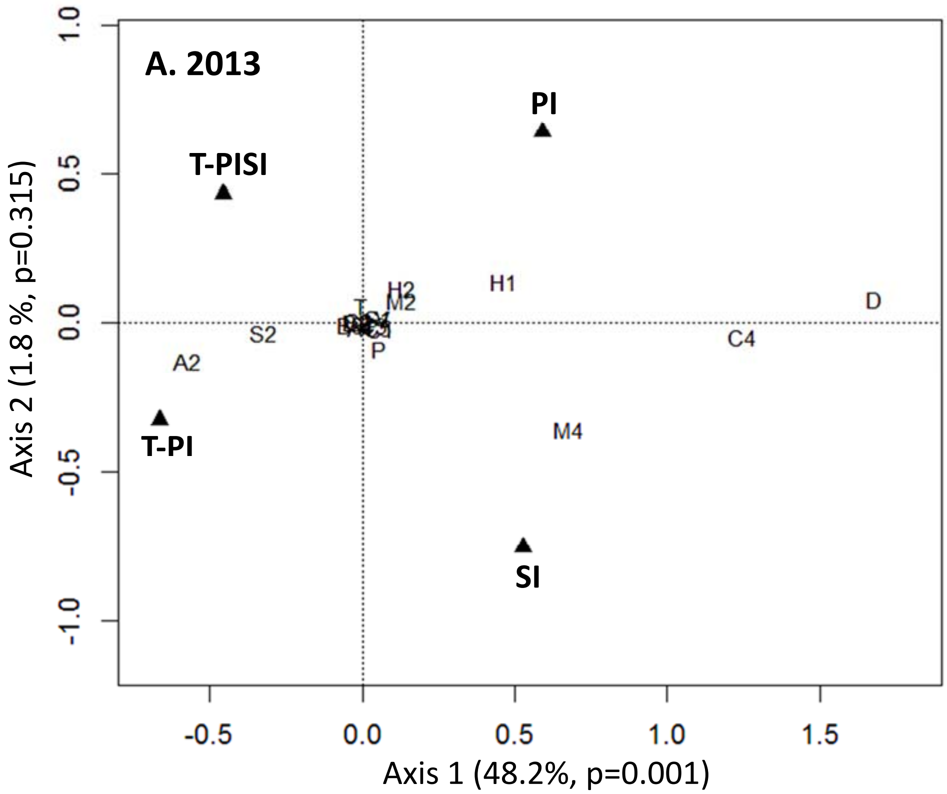



Figure 3. Relative proportion of abundance of insect foraging guilds at each practice. “Predator” represents predators/parasites/parasitoids foraging guild. Treatment: T-PI, OPI stand with no intercropped switchgrass; T-PISI, OPI stand with intercropped switchgrass; PI, YPI stand with no intercropped switchgrass; SI, YPI stand with intercropped switchgrass.


A. 2013
PI
T-PISI
T-PI
SI
H1
H2
M2
D
C4
S2
A2
P
M4
Axis 2 (1.8 %, p=0.315)
Axis 1 (48.2%, p=0.001)
1.0
0.5
0.0
-0.5
-1.0
-0.5
0.0
0.5
1.0
1.5

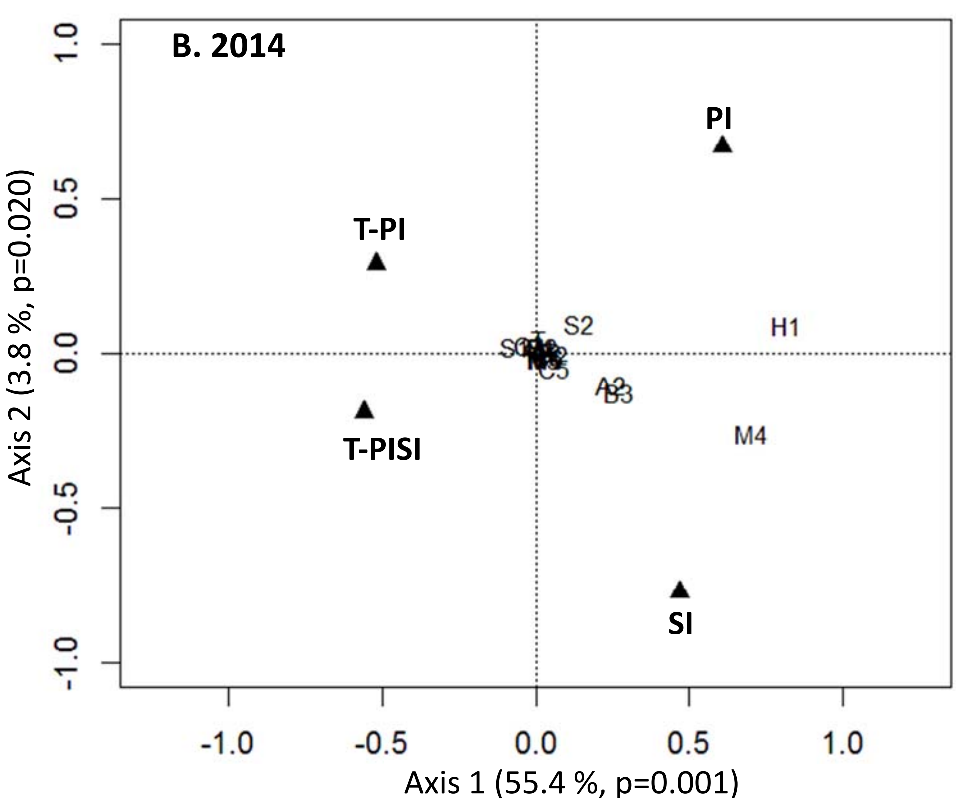

B. 2014
PI
T-PI
T-PISI
SI
S2
H1
C5
A2
B3
M4
Axis 2 (3.8 %, p=0.020)
Axis 1 (55.4 %, p=0.001)
1.0
0.5
0.0
-0.5
-1.0
-1.0
-0.5
0.0
0.5
1.0

Figure 4. Redundancy analysis (RDA) biplot of four practices and bee genera at Scooba site in 2013 (A) and 2014 (B). Variance (%) explained by each of the first two axis and *p* values calculated from permutation test of 999 iterations are shown in parenthesis. Treatment: T-PI, OPI stand with no intercropped switchgrass; T-PISI, OPI stand with intercropped switchgrass; PI, YPI stand with no intercropped switchgrass; SI, YPI stand with intercropped switchgrass. Family: A2, Apidae; B3, Buprestidae; C2, Cercopidae; C4, Cicadellidae; C5, Colletidae; D, Dolichopodidae; H1, Halictidae; H2, Hesperiidae; M4, Mordellidae; P, Pompilidae; S1, Sphecidae; S2, Syrphidae; T, Tabanidae. See Table 1 for names of other families, which are located at the center of the biplot.

Appendix A. Number of individuals of each family of insects detected at Scooba site, Mississippi during 2013-2014.

| Family | 2013 | | | | | 2014 | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| | May[1] | Jun | Jul | Aug | | May[1] | Jun[2] | Jul | Aug | |
| Alydidae[3,4] | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 1 | 1 |
| Andrenidae[4] | 5 | 0 | 1 | 0 | | 1 | 0 | 0 | 0 | 7 |
| Apidae | 37 | 226 | 115 | 33 | | 16 | 186 | 113 | 67 | 793 |
| Bombyliidae | 7 | 0 | 0 | 0 | | 11 | 0 | 0 | 1 | 19 |
| Buprestidae | 12 | 24 | 9 | 0 | | 19 | 83 | 8 | 1 | 156 |
| Cerambycidae | 9 | 3 | 0 | 0 | | 1 | 6 | 0 | 0 | 19 |
| Cercopidae | 2 | 1 | 0 | 2 | | 0 | 0 | 0 | 1 | 6 |
| Chrysididae | 0 | 0 | 4 | 2 | | 1 | 4 | 4 | 2 | 17 |
| Cicadellidae | 24 | 1001 | 730 | 318 | | 28 | 605 | 374 | 605 | 3685 |
| Colletidae | 1 | 8 | 1 | 0 | | 0 | 5 | 1 | 6 | 22 |
| Coreidae[3,4] | 0 | 0 | 0 | 0 | | 1 | 0 | 1 | 0 | 2 |
| Crabronidae[3] | 0 | 0 | 2 | 0 | | 0 | 1 | 0 | 0 | 3 |
| Dolichopodidae | 1462 | 805 | 449 | 488 | | 659 | 753 | 643 | 359 | 5618 |
| Halictidae | 79 | 198 | 131 | 164 | | 19 | 428 | 210 | 445 | 1674 |
| Hesperiidae | 6 | 36 | 75 | 11 | | 3 | 16 | 11 | 19 | 177 |
| Megachilidae[3] | 2 | 0 | 0 | 1 | | 0 | 8 | 4 | 0 | 15 |
| Meloidae | 2 | 10 | 12 | 6 | | 1 | 2 | 1 | 2 | 36 |
| Membracidae[3] | 0 | 0 | 0 | 0 | | 1 | 1 | 0 | 7 | 9 |
| Mordellidae | 314 | 788 | 151 | 38 | | 62 | 323 | 80 | 22 | 1778 |
| Pompilidae | 0 | 0 | 23 | 5 | | 1 | 2 | 3 | 4 | 38 |
| Reduviidae[3,4] | 0 | 0 | 0 | 0 | | 0 | 2 | 2 | 0 | 4 |
| Satyridae[3,4] | 0 | 0 | 0 | 0 | | 0 | 0 | 1 | 1 | 2 |
| Scoliidae[3,4] | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 1 | 1 |
| Sphecidae | 4 | 6 | 3 | 1 | | 2 | 15 | 5 | 5 | 41 |
| Sphingidae[3,4] | 1 | 1 | 0 | 0 | | 0 | 0 | 0 | 0 | 2 |
| Syrphidae | 12 | 43 | 219 | 430 | | 2 | 209 | 208 | 235 | 1358 |
| Tabanidae | 7 | 22 | 1 | 2 | | 3 | 13 | 4 | 3 | 55 |
| Tiphiidae[3,4] | 0 | 0 | 1 | 1 | | 0 | 6 | 0 | 0 | 8 |
| Vespidae | 4 | 5 | 6 | 2 | | 0 | 5 | 1 | 2 | 25 |
| **Total** | 1990 | 3177 | 1933 | 1504 | | 831 | 2673 | 1674 | 1789 | 15571 |

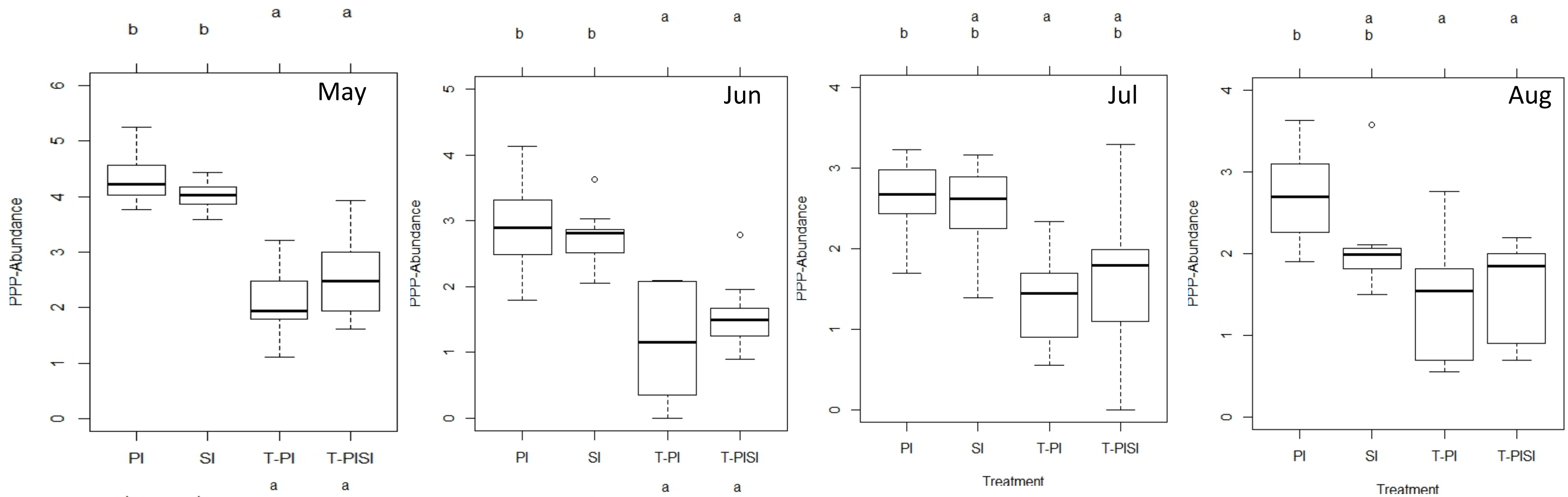
[1] One sampling occasion

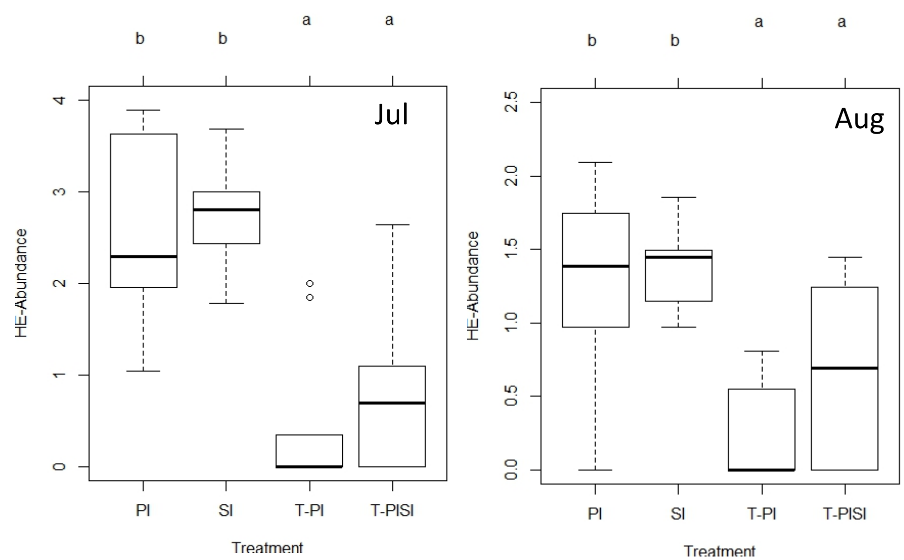
[2] Three sampling occasions

[3] Families captured <4 sampling stations in 2013

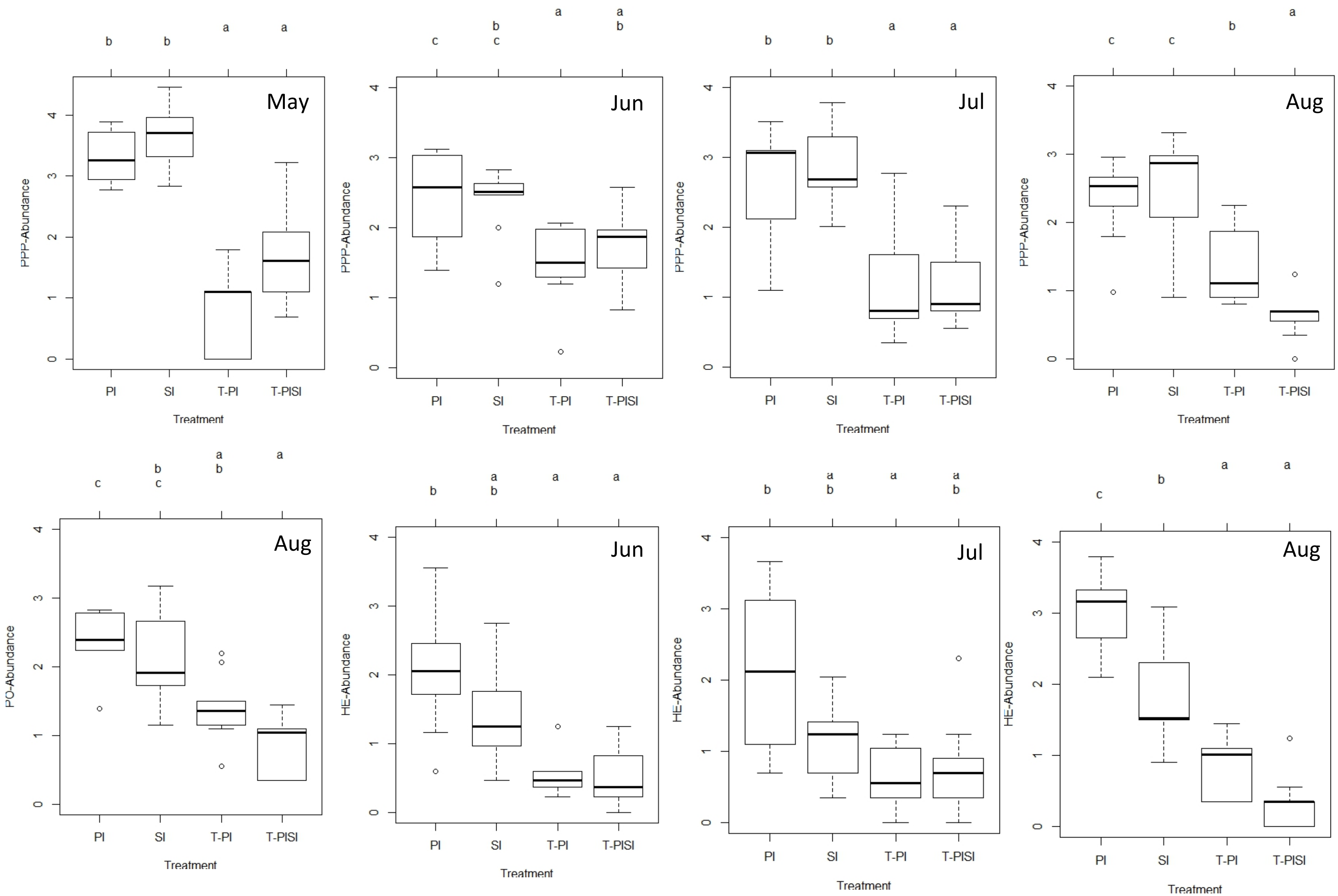
[4] Families captured <4 sampling stations in 2014

Appendix B. Multiple comparisons (Tukey's HSD test) of abundance of insects (predator/parasite/parasitoid, PPP and herbivore, H) between treatments in 2013. Treatments with the same letter (a or b) are not significantly different. Areas within a box are 25% quantiles and whiskers indicate the range of the data. Treatment: T-PI, OPI stand with no intercropped switchgrass; T-PISI, OPI stand with intercropped switchgrass; PI, YPI stand with no intercropped switchgrass; SI, YPI stand with intercropped switchgrass.



Appendix C. Multiple comparisons (Tukey's HSD test) of abundance of insects (predator/parasite/parasitoid, PPP and herbivore, H) between treatments in 2014. Treatments with the same letter (a, b, or c) are not significantly different. Areas within a box are 25% quantiles and whiskers indicate the range of the data. Treatment: T-PI, OPI stand with no intercropped switchgrass; T-PISI, OPI stand with intercropped switchgrass; PI, YPI stand with no intercropped switchgrass; SI, YPI stand with intercropped switchgrass.



Appendix D. Multiple comparisons (Tukey's HSD test) of family-level evenness between treatments in 2013. Only months that overall treatment effect was significant are shown (See Table 2 for overall treatment effect). Note that there was no significant treatment effect on evenness in 2014. Treatments with the same letter (a or b) are not significantly different. Areas within a box are 25% quantiles and whiskers indicate the range of the data. Treatment: T-PI, OPI stand with no intercropped switchgrass; T-PISI, OPI stand with intercropped switchgrass; PI, YPI stand with no intercropped switchgrass; SI, YPI stand with intercropped switchgrass.