THE IMPORTANCE OF SURFACE MINING AS A LANDSCAPE VARIABLE IN SHAPING WILD BEE COMMUNITIES IN CENTRAL APPALACHIAN FORESTS

BY

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Submitted to the Faculty of the Graduate School of Eastern Kentucky University in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2020

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ACKNOWLEDGEMENTS

Funding for this project was provided by the Steele-Reese Foundation and Eastern Kentucky University. We would like to thank the following 11 schools, their principals, and the participating teachers and students for their help in this project: London Elementary School, 4th grade; Paces Creek Elementary 3rd and 4th grade; Mountain View Elementary, 4th and 5th grade; Emmalena Elementary, middle grades; A.B. Combs Elementary, middle grades; Arlie Boggs Elementary, middle grades; Beaver Creek Elementary, $3rd$ and $4th$ grade; Hazard High School, $9th$ grade; Fleming-Neon Middle School; Martha Jane Potter Elementary, $4th$ and $5th$ grade; and Leatherwood Elementary, middle grades. We would also like to thank field assistants Kathryn Tucker and Benjamin Slusher, as well as NSF REU Grant #1559980 student Hannah Carter for their help in collecting specimens, processing specimens, and working with students.

ABSTRACT

Anthropogenic disturbance has led to widespread losses in biodiversity. Native bees (Hymenoptera, Apoidea) provide essential pollination services and have been declining in abundance dramatically in recent years. One potential cause of these declines is anthropogenic disturbance. Eleven sites were established in Southeastern Kentucky, U.S.A. where surface mining for coal is common. Data was analyzed using generalized linear mixed-effect models to detect the most important landscape scale variables, including mining, in shaping bee communities. Bee species richness was significantly lower on sites that contained surface mines, but abundance was similar between mined and unmined sites. The proportion of each nesting group from each site was not significantly influenced by landscape variables in most cases. Cavity nesting bees, however, were most strongly influenced by the presence of a mine as well as the percent herbaceous cover and percent forest cover.

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Chapter I: The importance of surface mining as a landscape variable in shaping bee communities of Central Appalachian forests.

Introduction

Native bees serve as pollinators for a wide variety of both native plants and crop species (Potts et al. 2003). Native bees can be specialists or generalists, and some plant species are exclusively pollinated by native bee species (Fowler, 2016; Potts et al., 2010). Native bees have also been shown to mediate the reduction in pollination services that has resulted from the decreasing numbers of honey bees, *Apis mellifera* (Winfree, Williams, Dushoff, & Kremen, 2007). The population status of many native bee species has not been well studied, but data on some groups such as the bumblebees, *Bombus spp.*, have shown declines. These declines are most likely linked to climate change, habitat loss and fragmentation, and use of insecticides (Cane, 2001; Potts et al., 2010).

Surface mining for coal contributes to the loss of natural habitats throughout large portions of the Appalachian Mountains (Ross, Mcglynn, & Bernhardt, 2016). Some practices, such as mountain top removal mining, have secondary environmental impacts in addition to natural habitat loss. This is because the overburden, or materials removed from the area to be mined, is relocated to a nearby valley, or held off site during the mining process (Palmer et al., 2010). Thus habitat loss is compounded both from vegetation that is removed during mountaintop removal and vegetation in the valley that is covered by overburden materials (Palmer et al., 2010). After the coal has been extracted, reclamation efforts are legally mandated, but despite this, surface mined areas are rarely restored to their original state (Zipper et al., 2011). Under the Surface Mining Control and Reclamation Act of 1977 (SMCRA) reclamation efforts ought to restore

affected land to the original or improved conditions (Surface Mining Control and Reclamation Act of 1977, 2017). Part of the reclamation process includes replacing overburden, which is often heavily compacted to avoid erosion and landslides (Randall et al. 1978, Surface Mining Control and Reclamation Act of 1977, 2017). The compacted overburden is overall of low quality as a growing medium for vegetation due to heavy compaction, loss of natural soil horizons, and inadequate organic materials (Acton et al., 2011). Grasses are usually planted early in reclamation to satisfy the requirement of SMCRA to establish a self-regenerating vegetative cover equal to that of the pre-mining landscape (Yeiser et al. 2016, Surface Mining Control and Reclamation Act of 1977, 2017). Grasses are usually the easiest form of vegetative cover to establish, but do little to form a healthy soil (Acton et al., 2011). Reforestation and the establishment of woody shrubs is sometimes attempted, but is often unsuccessful, especially early in the reclamation process due to the high compaction and low soil quality (Franklin et al. 2012). The planting of grasses during reclamation and the difficulty of establishing trees leads to a shift from a diverse deciduous forest habitat to a grassland low in plant species richness (Larkin et al., 2008).

Habitat loss and degradation of the local environment associated with mountaintop mining is often therefore unresolved with reclamation efforts (J. Wickham et al., 2013) and may have long-term impacts to local biodiversity. For instance, lower species richness of benthic macroinvertebrates and lower species abundance of salamanders were found in streams impacted by valley fills (Price et al., 2018; U.S. Environmental Protection Agency (EPA), 2005). The abundance of birds and salamanders dependent on the habitat provided by the mixed mesophytic forest also

declined with the removal and fragmentation of forests from surface mining (Price et al., 2018; J. Wickham et al., 2013). Similarly, small mammal species diversity on previously mined sites was much lower than that of nearby forest and grassland habitat, likely resulting from the unsuitable habitat for forest species and low connectivity to source populations for grassland species (Larkin et al., 2008).

Although it is generally understood that biodiversity will be negatively impacted by the presence of surface mining in the local environment (EPA 2005, Larkin et al. 2008, Wickham et al. 2013), few studies have evaluated the effects of surface mining on bee biodiversity specifically. Two recent studies focused on the capacity of reclaimed mines to support bumblebee populations (Lanterman & Goodell, 2018) and overall bee diversity (Cusser & Goodell, 2013). Both studies indicate that floral diversity and the proportion of forest in the surrounding landscape play a significant role in whether the reclaimed mine can support healthy bee populations (Cusser & Goodell, 2013; Lanterman & Goodell, 2018). Higher quality reclaimed mines for bees had invested significant amounts of money into the reclamation process or were sites that had allowed sufficient time for natural succession to return high quality bee forage plants that were available throughout the foraging season of bees (e.g. >20 years without disturbance) (Lanterman & Goodell, 2018).

Although both studies considered the contribution of forest proximity in their analysis, our study builds upon this work to understand how the proportion of surface mining in the landscape influences bee communities, and the relative importance of this landscape variable compared to other land use types. Given the far-reaching and secondary environmental impacts of surface mining, we predicted that even low levels of

surface mining in a landscape would have a greater impact on bees than other land use types that are associated with biodiversity loss, e.g. developed land.

The objective of our study was to quantify the impact that surface mining in the landscape has on bee communities. Specifically, we aim to understand how bee species richness, abundance, and functional diversity are affected by the amount of land cover dedicated to reclaimed and active surface mines in a landscape, and to empirically test how this landscape-level variable compares to other land cover categories in terms of their effect on bee communities. To understand how bee communities and ecosystem function may be impacted by surface mining we assigned bees to functional groups. We assigned functional groups based on nesting habit because this trait shows consistent responses in the sensitivity of species to environmental change (Williams et al., 2010). Bee functional groups in our study were classified into the following five groups, based on nesting behavior: cleptoparasites, ground-nesters, hive nesters, stem nesters, and cavity nesters. We predicted that different bee functional groups would respond differently to the amount of surface mines in a landscape. For example, although ground nesting bees are less affected by most human induced environmental change compared to above ground nesting bees (Williams et al., 2010), we predicted that ground nesting bees would occur at lower numbers in landscapes with higher amounts of surface mines owing to the high proportion of compacted ground in the landscape from mining practices, Additionally, we predicted that hive nesting species, such as wild honey bees may benefit from the wild flowers and grasses that colonize the early successional habitats of surface mines.

Methods

Study area

Southeastern Kentucky (SE KY), with approximately 150 active surface mines (Kentucky Energy and Environment Cabinet & Department for Energy Development and Independence, 2016)is the ideal location to understand how active and recently reclaimed surface mining may impact bee biodiversity. Six contiguous rural counties in SE KY with active surface mining were selected for the study including Laurel, Clay, Knox, Leslie, Letcher, and Perry County (Figure 1). The counties selected have between 57.27% and 88.55% forest cover, with all but one county (Laurel) having above 75% forest cover (USDA National Agricultural Statistics Service Cropland Data Layer, 2017). Active surface mines and some reclaimed surface mines in SE KY can pose substantial risk for direct sampling on the site due the steep gradients. Therefore, sampling locations for the study were selected as K-12 schools embedded in landscapes that either contained surface mines or were without surface mining. We elected to sample on K-12 schools within the landscapes due to the ease of access to the grounds and to allow for participation of the students in the project. Over 300 students who were included in the project received an approximately 45 min to 1-hour lecture on pollinators and bee diversity.

Pre-selection of sites involved locating all K-12 schools in each of the six SE KY counties and creating a 1000m buffer around each school using ArcMap 10.3. While bees' maximum foraging ranges are often positively correlated with body size, typical foraging distances are usually much shorter than the maximums recorded for those species (Gathmann, Tscharntke, Journal, & Sep, 2002; Greenleaf, Williams, Winfree, & Kremen, 2007), (Gathmann et al., 2002; Sardiñas, Tom, Ponisio, Rominger, & Kremen,

2016; Zurbuchen et al., 2010). Actual foraging distances are largely dependent on the surrounding landscape and resource availability, with most foraging activity occurring in close proximity to the nest (Gathmann et al., 2002; Sardiñas et al., 2016; Zurbuchen et al., 2010). Therefore, it was a reasonable assumption that the majority of bees captured near the center of the sampling buffer were only nesting and foraging within the 1000m radius (Gathmann et al., 2002; Greenleaf et al., 2007; Sardiñas et al., 2016; Zurbuchen et al., 2010).

Figure 1. Map of research sites and surface mines in Southeastern Kentucky. Mined sites are shown in black, research sites with mines in dark gray, and unmined research sites in light gray.

Next a layer including all surface mines in the six counties (Commonwealth of Kentucky, 2011) was added to the map. The map was visually scanned for areas where the mines did and did not overlap the 1000m buffer. Final site selection met the following two criteria (1) sites were separated by at least 2000m, and (2) an equal proportion of the sites were with and without surface mines within the 1000m buffer. Final permission for sampling on the school grounds was obtained for a total of eleven sites: seven schools containing a surface mine within a 1000m radius and four schools without any active or reclaimed mining operations within the 1000 m buffer.

The areas available at most sites were recreational fields that were often used by students. One site provided an area of 50m by 50m that remained un-mowed and undisturbed by students for the duration of the experiment. One other site was un-mowed after late May in the year sampling took place. All other sites were mowed regularly but were bordered by unmanaged forest or herbaceous vegetation. All areas where sampling took place were under $500m^2$. The most common types of vegetation in these landscapes consisted of common turf grasses, white clover (*Trifolium repens*) and other legumes in the genus *Trifolium*, invasive shrubs such as Japanese honeysuckle (*Lonicera japonica)*, multiflora rose (*Rosa multiflora)*, and other flowering plants common to recreational areas.

Bee surveys

Bee communities were sampled using both active and passive methods. Sampling occurred over three major periods: spring (March 15, 2017-May 31, 2017), summer (June 1, 2017-August 31, 2017), and fall (September 1, 2017-October 31, 2017). UV blue, UV yellow, and white pan traps were deployed for a 24-48 hour period for up to three times per sampling season. Thirty pan traps were placed along a 150m transect at a distance of 5m apart alternating colors along the transect. The three colors used simulate UV pollen guides found on flower corollas and each color attracts different portions of the bee community (Geroff, Gibbs, & McCravy, 2014). Pan traps were filled approximately ¾ of the way full with a soapy water solution (Droege, 2008).

Blue and yellow vane traps were hung from selected points (often fences or woody vegetation) in the sampling area. Vane traps were filled about 34 of the way full with a soapy water solution (Droege, 2008) and were left out for 5-7 days unless

inclement weather occurred. One trap of each color was deployed at least once per sampling period. Netting was also performed for 30 minutes at the time vane and pan traps were set, weather permitting. Net sampling has been shown to out-perform trap collecting in regards to species richness, but requires greater sampling effort (Popic et al. 2013).

It was our goal to perform at least one sample of each type once per sampling season at each site. However, this goal was only achieved for each site during the summer sampling period when schools were not participating in the data collection. Spring and fall samples were conducted in participation with teachers and students at the schools which led to some missing samples during these seasons. Additionally, one school participating in the experiment was permanently closed in May 2017, preventing a fall sample. The number of samples per season per site ranged from 0-10 in the Spring, 1- 3 in the Summer, and 0-4 in the Fall (Appendix A).

All specimens collected in the field were temporarily stored in 70% ethyl alcohol. Bees were separated from other insects in the sample and were placed in mesh bags. The bees were then washed to remove debris such as pollen and plant material to aid in identification. Each sample was washed once with hot water and Dawn blue dish soap, shaken for 5 minutes, rinsed in hot water for 5 minutes, rinsed in cold water for 2 minutes, and briefly rinsed in 70% ethanol. Small bees with few hairs, such as *Ceratina spp.* and *Lasioglossum spp.*, were then removed from the bags and patted dry with a paper towel. Larger, hairier bees were placed in a covered plastic hamster wheel and dried with an 1875-Watt hair dryer on high heat until all hairs were dry. Cleaned specimens were immediately pinned and labeled.

Identification and Functional Group Classification

All specimens were identified to genus or species level using morphological characteristics. Each species was then assigned several functional traits based on its nesting habit: ground, cavity, stem, hive, and cleptoparasite (Baker, Kuhn, & Bambara, 1985; Cane, 1991; S. Colla et al., 2011; Eickwort, 1981; Fowler, 2016; Gonzalez & Griswold, 2013; Graham, Willcox, & Ellis, 2015; Johnson, 1981; Michener, 2007; Normandin, Vereecken, Buddle, & Fournier, 2017; Ordway, 1966; Osgood, 1972; Rehan & Richards, 2010; Stockhammer, 1966). Honeybees and *Bombus spp.* were categorized as social bees, while others were considered solitary (Michener, 2007). If natural history information was not available at the species level, then genus level natural history information was used.

Land cover classification

Percent land cover for each 1000m buffer was calculated using data from the 2011 National Landcover Database (NLCD 2011)(U.S. Geological Survey, 2016). The landcover classifications were simplified into the following 8 classes: herbaceous, forest, cropland, developed, barren, shrub, open water, and wetlands. The NLCD 2011 raster file was loaded into ArcMap 10.3 and then converted to polygons. Then a layer was created using the union function to combine the buffer layer and the NLCD 2011 polygons. The attribute table of this union layer was then exported and each landcover class was appropriately coded. The area of each land cover polygon was divided by the total area of the buffer to calculate the percent land cover of that polygon. Then each land cover class was totaled for each site to make up the percent land cover for each site (Table 1). The

same process was performed using the mine layer maps for mined sites to calculate the percent of mined area.

Statistical analysis

Species richness, abundance, and functional groups were analyzed with respect to landscape scale factors using general and generalized linear mixed models. Models for species richness used a negative binomial error distribution to allow for overdispersion. Abundance models were log transformed, because less overdispersion was present in this response variable. All functional group responses were true proportions, so all models of nesting group responses were square root transformed to meet the conditions of normality. The following land cover variables were included as predictor variables in the models: percent mine cover, mine presence/absence, percent forest cover, percent herbaceous cover, and percent developed cover. Percent cover for agricultural, shrubland, and barren land were also calculated, but did not appear at all sites and comprised very little of the land cover so were not used in the analysis. Developed cover and forest cover were not included in the same models because they were highly correlated with each other (Pearson product moment correlation =0.993). A candidate set of twelve models were selected. The candidate set included single factor models of all predictor variables and the following two factor models: Mine presence/absence + Forest cover; Mine cover + Herbaceous cover; Herbaceous cover + Forest cover; Herbaceous cover + Forest cover; Mine presence/absence + Herbaceous cover; Mine cover + Developed cover as well as a null model only included a random intercept. Site was included as a random effect in all candidate models.

Site	Mine	Forest	Developed	Herbaceous	Crop	Shrub	Barren
	$\frac{0}{0}$	$\%$	$\%$	$\%$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{0}{0}$
AB Combs	0.00	51.96	39.61	7.98	0.00	0.00	0.31
Arlie Boggs	0.00	66.65	14.99	17.70	0.34	0.00	0.32
Beaver Creek	6.23	75.10	8.92	11.79	3.94	0.03	0.23
Emmalena	1.43	75.55	9.90	13.45	0.00	0.26	0.84
Fleming-	0.61	72.64	21.92	5.15	0.00	0.00	0.29
Neon							
Hazard High	5.75	44.47	52.08	1.34	0.00	0.26	1.28
Leatherwood	5.21	86.38	7.53	3.66	0.00	0.00	2.43
London	0.00	13.15	81.33	1.55	3.52	0.00	0.46
Martha Jane	0.42	76.31	14.64	7.06	0.00	0.00	1.99
Potter							
Mountain	0.00	79.09	11.30	6.43	0.00	0.00	3.18
View							
Paces Creek	4.05	42.78	51.53	2.85	2.84	0.00	0.00

Table 1. Landcover attributes of sample sites, values in percent of total area.

The best models for abundance, species richness, and functional groups were selected using Akaike's Information Criterion (AIC). The lowest AIC was selected as the top model. Models were considered as competing models if their ΔAIC <2.0 (Burnham, Anderson, & Burnham, 2002).

Results

A total of 710 bees were collected comprising 100 species from 36 genera. Appendix B summarizes the species collected from sites with and without surface mining in the landscape. Species accumulation curves indicate that sampling effort was sufficient for the bee communities at both site types (Figure 2). Bee species richness after accounting for abundance was significantly lower on sites with surface mines within the landscape compared to sites without surface mines within the landscape, according to the rarefaction curves rescaled by individuals with 95% CI (Figure 3) The best model for bee species richness, with 37% support, included a negative effect of mine presence in the landscape (Table 2, Figure 4).

Figure 2. Species accumulation curves for mined and unmined sites.

Figure 3. Rarefaction curves for mined and unmined sites.

Explanatory	Bee Abundance			Bee Species Richness				
Variables								
	AIC	$\triangle AIC$	AIC	AIC	$\triangle AIC$	AIC		
			Weight			Weight		
Mine %	185.02	5.42	0.03	327.76	4.2	0.05		
Mine Presence	183.41	3.81	0.07	323.56	$\bf{0}$	0.37		
Mine Presence +	185.49	5.89	0.02	325.33	1.76	0.15		
Forest %								
Forest %	185.37	5.77	0.03	327.02	3.45	0.07		
Developed %	186.03	6.43	0.02	328.08	4.52	0.04		
Mine $% +$ Herbaceous	185.34	5.74	0.03	330.15	6.58	0.01		
$\%$								
Herbaceous $% +$	179.6	$\bf{0}$	0.47	327.52	3.95	0.25		
Forest %								
Herbaceous %	183.39	3.79	0.07	329.35	2.37	0.02		
Herbaceous $% +$	182.01	2.41	0.14	327.62	4.05	0.08		
Forest $% +$ Mine								
Presence								
Mine Presence +	184.11	4.51	0.05	325.93	2.37	0.05		
Herbaceous %								
Null	184.05	4.44	0.05	327.24	3.68	0.06		
Mine $% +$ Developed	187	7.4	0.01	328.69	5.13	0.03		
$\%$								

Table 2. Summary of models with bee abundance or species richness as response variables and landscape variables as explanatory variables. The best models for each response variable (ΔAIC<2) are in bold.

Figure 4. Species richness and abundance for mined and unmined sites +\- standard error.

There was only one competing model in the candidate set for bee species richness. The competing model had 15% weight of evidence and included a negative effect of mine presence in the landscape and a negative effect of the percent cover of forest in the landscape (Table 2) (Figure 5).

Species abundance was higher on unmined sites than on mined sites (Figure 4), but mining was not a significant factor in determining bee abundance according to our models (Table 2). The model with the greatest support included the percentage of herbaceous and forest cover with 47% weight of evidence (Table 2). There were no competing models for species abundance.

Different functional groups exhibited somewhat differing responses to land cover characteristics (Figure 6). The best model for cavity nesting bees with 42% support included the presence of a mine in the landscape and the percent cover of herbaceous land (Table 3). Both land cover variables had a negative effect on the abundance of cavity nesting bees. There were no competing models for cavity nesting bees.

The best model for stem nesting bees included only a negative effect of the presence of surface mining in a landscape (18% weight of evidence; Table 3). Seven competing models were identified (Table 3). The null model was identified as the best model for the following functional groups: hive nesting bees, ground nesting bees and cleptoparasitic bees.

Figure 5. Functional group responses to the presence of a mine in the 1000m buffer. The functional groups shown are as follows: A. Ground nesters, B. Hive nesters, C. Cavity nesters, D. Stem nesters. Unmined sites are designated by "no" on the left of the x-axis, while mined sites are designated by "yes" on the right side of the x-axis.

μ . μ , μ , μ , μ , μ Nest Habit	Explanatory Variable			
				AIC
		AICc	$\triangle AIC$	weight
Ground	Mine presence	329.45	2.3	0.1
	Mine %	329.45	2.3	0.1
	Forest %	329.43	2.28	0.1
	Developed %	329.44	2.29	0.1
	Herbaceous %	329.37	2.22	0.11
	Mine + Forest%	331.8	4.65	0.03
	Mine + Herbaceous%	331.76	4.61	0.03
	Mine $+$ Herbaceous%			
	$+Forest%$	334.23	7.08	0.01
	Mine%+Developed%	331.82	4.67	0.03
	Mine%+ Herbaceous%	331.75	4.6	0.03
	Herbaceous%+Forest%	331.76	4.6	0.03
	NULL	327.15	$\boldsymbol{0}$	0.32
Stem	Mine presence	292.15	$\boldsymbol{0}$	0.18
	Mine %	294.01	1.86	0.07
	Forest %	292.86	0.72	0.12
	Developed %	292.94	0.8	0.12
	Herbaceous %	295.83	3.69	0.03
	$Mine + Forest\%$	293.65	1.51	0.08
	Mine + Herbaceous%	292.9	0.76	0.12
	Mine $+$ Herbaceous%			
	$+Forest%$	295.37	3.23	0.03
	Mine%+Developed%	293.8	1.65	0.08
	Mine%+ Herbaceous%	293.74	1.6	0.08
	Herbaceous%+Forest%	295.24	3.09	0.04
	NULL	294.45	2.3	0.06
Hive	Mine presence	317.35	1.02	0.14
	Mine %	317.43	1.11	0.14
	Forest %	318.15	1.83	0.09
	Developed %	318.14	1.82	0.1
	Herbaceous %	318.58	2.25	0.08
	Mine + Forest%	319.73	3.41	0.04
	Mine + Herbaceous%	319.69	3.37	0.04
	Mine $+$ Herbaceous%			
	$+Forest%$	322.11	4.17	0.03
	Mine%+ Developed%	319.71	3.39	0.04
	Mine%+ Herbaceous%	319.66	3.34	0.04

Table 3. Summary of models with bee nesting habits as response variables and landscape variables as explanatory variables. The best models for each response variable (ΔAIC<2) are in bold.

Table 4 (continued).

Discussion

The findings of our study highlight the important effect that surface mining at the landscape scale has for bee diversity and ecosystem function. While other landscape variables also played a role in shaping bee communities in our study area, the presence of surface mining in the landscape was consistently included within the set of top models for most of the bee response variables we tested.

The negative environmental impacts and degree of forest loss related to surface mining are well documented (Haering, Daniels, & Galbraith, 2004; Larkin et al., 2008; Randall et al., 1978; Ross et al., 2016; U.S. Environmental Protection Agency (EPA), 2011; Zipper et al., 2011). However, the consequences of and specific factors influencing many groups, even many locally endangered species, related to surface mining has not received sufficient study (Yeiser et al., 2016; Zipper et al., 2011). Further study is required to determine the specific reasons why surface mining negatively impacts bee communities. Some possible hypotheses include: decrease in landscape connectivity (Larkin et al., 2008; J. Wickham et al., 2013), changes in and quality of the vegetative community (Acton et al., 2011; Franklin et al., 2012; Yeiser et al., 2016; Zipper et al., 2011), and impacts related to water quality (Palmer et al., 2010; Ross et al., 2016; U.S. Environmental Protection Agency (EPA), 2005).

Surface mining is one of the most influential sources of land use change and fragmentation in Southeastern Kentucky and the Interior Appalachian Plateau ecoregion (Drummond & Loveland, 2010). Habitat fragmentation and loss of forest habitat have been shown to have negative impacts on functional diversity and species richness of bee communities (Martins, Gonzalez, & Lechowicz, 2015; Potts et al., 2010; Rathcke &

Jules, 1993). Therefore, it is worth investigating if bee communities more greatly impacted by the habitat fragmentation or another impact of surface mining.

Surface mining has the potential to radically change the vegetative community. These changes are three-fold: loss of vegetation occurring at the highest and lowest elevation scales due to mountain top removal and valley fills (J. Wickham et al., 2013), loss of pre-existing herbaceous and forest cover during mining operations (Franklin et al., 2012; J. Wickham et al., 2013; Yeiser et al., 2016; Zipper et al., 2011), and the plants and methods used during reclamation (Franklin et al., 2012; Yeiser et al., 2016; Zipper et al., 2011). The loss of topographic complexity due to mountain top removal and valley fills results in a shift from vegetative communities occurring at low to high elevations to a dominance of vegetative communities occurring at middle elevations (J. Wickham et al., 2013). This leads to an overall reduction in plant species richness and has been shown to have cascading impacts on animals dependent on elevation limited species, such as the Cerulean Warbler, *Dendrocica cerulea* (U.S. Environmental Protection Agency (EPA), 2005; J. Wickham et al., 2013). It is currently unknown if the plants impacted by loss of topographic complexity have dependent insect species. During surface mining operations all vegetation, both woody and herbaceous, must be removed from the area leading to a drastic and sudden decline in plant species richness and a large area unusable to pollinators. The impact on pollinators directly resulting from this initial vegetation removal both from the removal site and neighboring areas needs further study. Lastly, the vegetative community that is re-established after mining operations have concluded is vastly different than the pre-existing vegetative community. Exotic and invasive species are often planted during reclamation operations due to their competitive ability, easy

establishment, and nutrient sequestration abilities (Franklin et al., 2012; Yeiser et al., 2016; Zipper et al., 2011). These reclamation practices are especially common in post-SMCRA mine sites and may inhibit the growth of woody vegetation and secondary succession (Franklin et al., 2012). Studies that have compared typical to ecologically focused reclamation practices have largely focused on birds and mammals, so the impact on pollinators is unknown (Yeiser et al., 2016).

Our study supports other studies that have found a negative response of the bee community to the proportion of forest in the landscape (Winfree, Aguilar, Vazquez, Lebuhn, & Aizen, 2009; Winfree, Williams, Gaines, Ascher, & Kremen, 2008; Winfree, Griswold, & Kremen, 2007). However, recent evidence shows distinct communities of bees between forests and anthropogenic land uses in areas where temperate forests were the dominant land use type prior to urbanization and conversion of forests to agriculture (Harrison et al. 2018). Having some forested area in the landscape may increase species richness and abundance by protecting forest specialist bee species as well as providing critical nesting habitat for bee species that are not forest specialists (Bennett & Isaacs, 2014; Cane, 2001; Quintero, Morales, & Aizen, 2009; Svensson, Lagerlöf, & G. Svensson, 2000) with this increase becoming less evident with distance from forest (Cusser & Goodell, 2013). It is also likely that forest specialist bees, including those bees that forage primarily on early season flowers characteristic of temperate forest phenology (Harrison et al. 2018) either were not collected due to the timing of our sampling or were less frequently collected in the study area because of forest loss in the sampled sites. These forest specialist bees are at peak abundance in the early spring before canopy leafout, which occurs in March and April in Southeastern Kentucky (Grundel et al., 2010; Taki, Kevan, & Ascher, 2007).

It is also possible that these bees were largely absent from our sample due to edge effects. Surface mining in Central Appalachia contributes to forest loss, with the majority of the losses occurring in the interior forests (J. D. Wickham, Riitters, Wade, Coan, & Homer, 2007). This leads to an increased amount of edge habitat, which has a different plant community and ecological function than interior forests (J. D. Wickham et al., 2007). These edges have greater numbers of exotic species and fewer shade-tolerant plants, which may be important pollen or nectar sources for bees (J. D. Wickham et al., 2007).

Herbaceous land cover generally provides bees with floral diversity and appropriate forage and may be an important predictor of community composition (Bennett & Isaacs, 2014; Lowenstein, Matteson, Xiao, Silva, & Minor, 2014; Svensson et al., 2000). Herbaceous cover generally has a positive overall effect on species richness and abundance (Bennett & Isaacs, 2014). Bee species richness and abundance declined in a similar fashion in association with mining as with other anthropogenic disturbances (Quintero et al., 2009; Winfree et al., 2009; Winfree, Griswold, et al., 2007).

Bee responses to mining and land use change can be predicted by certain functional traits, especially nesting habit. Both in our study and others, cavity nesting bees have responded negatively to increased herbaceous land cover (Lowenstein et al., 2014). Cavity nesting bees have also been found to have a positive association with the presence forest cover in the landscape (Winfree, Griswold, et al., 2007). We found that ground nesting bees were less impacted by the presence of mining in the landscape (null model was the best model), which is consistent with findings regarding their responses to other types of disturbance (Williams et al., 2010).

We chose to conduct our analyses with a 1000m buffer based on the results of our analyses, the location of our sites, and the average flight distances of common species in the study area (Greenleaf et al., 2007). Many other studies have used a scale closer to 1500m, which is considered to be a fairly typical foraging distance (Bennett & Isaacs, 2014; Greenleaf et al., 2007; T. H. Ricketts et al., 2008; Winfree, Griswold, et al., 2007). However, differences in bee community composition have been detected at scales as small as within a 100m radius (Lowenstein et al., 2014).

Species richness and abundance are both necessary measures for making conservation and management decisions. This study provides baseline data regarding bee communities in the heavily mined Southeastern Kentucky area of the Appalachian Mountains. Additionally, very few bee surveys have been conducted in SE Kentucky. The only documented survey from the area, with more than 50 specimens since 1990, was conducted from 2007-2011 by the USGS (Droege and Sellers 2017). More data regarding species records and natural history information is needed to target management goals and species of greatest concern. Museum collections and dated, georeferenced collection data has been instrumental in the providing quantitative evidence of bumblebee declines in species such as *Bombus affinis* (S. R. Colla & Packer, 2008). Further data collected over many years could provide evidence of temporal changes in species and communities. This may be integral to identifying declines in species and groups that receive less research and conservation effort.

Conclusion

We found that the bee communities sampled from schoolyards in Appalachia are negatively impacted by surface mining and high forest cover in the landscape, while they are positively impacted by herbaceous land cover. This information can be used to inform management practices of formerly mined areas and when selecting future sites for mining.

Chapter II: Surface mined landscapes host distinct bee communities and less diverse plant-pollinator networks.

Abstract

Surface coal mining in Central Appalachia typically involves the clearing of all native vegetation, the removal of large amounts of sediment to access seams of coal, and the subsequent deposition of removed sediment into adjacent valleys. This form of mining, even for small-scale operations, results in major topographical changes, dramatically altering entire landscapes. Current reclamation practices often involve the compaction of soils and planting of non-native plant species. Despite the fact that surface mining results in permanent changes to the landscape, few studies have evaluated the impact of surface mining on biodiversity at the landscape-scale. In particular, the landscape-scale impact of a permanently altered vegetative cover, may be especially important for the pollinator community of Appalachia, where some pollinators may potentially benefit from the permanent conversion of mature forest to grasslands. The aim of our study was to compare bee diversity between surface mined landscapes and landscapes that did not contain surface mines or reclaimed surface mines. A total of 710 bees representing 100 species were collected from 11 sites throughout southeastern KY. Although several landscape level factors were analyzed, constrained ordination found that the presence of surface mining in a landscape and the proportion of herbaceous cover in a landscape together explained 31% of the variation in bee community composition between sites $(F_{2,8}=1.798; p=0.02)$..

Key words: Appalachia, Apoidea, bees, land use change, mutualisms

Introduction

Central Appalachia has been historically dominated by the complex, highly diverse mixed-mesophytic forest ecosystem (Larkin et al., 2008). This ecosystem hosts remarkable levels of plant and animal endemism and biodiversity for a temperate forest (T. Ricketts et al., 1999). Surface coal mining in the Appalachian mountains has resulted in widespread topographic rearrangement and the landscape-scale permanent conversion of these diverse mature Appalachia forests to non-native grassland ecosystems (Wickham et al., 2013; Maigret et al., 2019). Surface coal mining typically involves the clearing of all native vegetation as well as the topsoil and subsoil layers, the removal of large amounts of sediment to access seams of coal, and the deposition of removed sediment into adjacent valleys (Palmer et al., 2010; Maigret et al., 2019). The most recent estimates of the extent of surface coal mining in Appalachia have found that 5700km² of forest had been removed and over 3200km of streams had been filled (EPA 2005).

In post-mined landscapes, reclamation efforts are often conducted to modify the otherwise unused landscapes to be utilized for agriculture, habitat restoration, and landscape development (Davis, 2006). The number of reclaimed mine sites are increasing in number due to the decreasing demand for mining operations (Davis, 2006). Current reclamation practices rarely restore native forest, but instead leave formerly mined lands with heavily compacted soils and dominated by a non-native, and predominately grassy vegetative cover (Zipper et al., 2011).

Inadequate restoration of formerly mined sites results in the widespread loss of mature Appalachia forest as well as the former topographic heterogeneity associated with the high biodiversity of Central Appalachia (Maigret et al. 2019). However, few studies

have quantified the impacts of this permanent and landscape-scale disturbance on terrestrial biodiversity or ecological processes. Pollinator communities may be especially affected by current surface mine reclamation practices, as pollinator community composition is heavily shaped by the vegetative community (Hatfield & LeBuhn, 2007; Potts et al., 2003). Specifically, the landscape-level conversion of mature Appalachia forest to grassland ecosystems may benefit some species of pollinators while negatively impacting other species. Because pollinators provide the important ecosystem service of pollination, it is important to understand not only the species level impacts on pollinators from surface mining in landscapes, but also whether changes in pollinator community composition are associated with changes in pollination services. The degradation of natural ecosystems can impact individual species as well as the interaction networks and food webs that the species comprise.

Methods

Study site

Southeastern Kentucky is the ideal region to understand how surface mining and reclaimed landscapes affect bee biodiversity due to its approximate 150 active coal surface mines (Kentucky Energy and Environmental Cabinet and Department of Energy Development and Independence, 2016). Eleven sites located in six contiguous counties: Perry, Leslie, Knott, Letcher, Harlan, Clay, and Laurel, in southeastern Kentucky were selected for this study (Figure 1). The counties selected ranged between 57.27% and 88.55% forest cover. Actively mined areas and some reclaimed mines in the region can pose substantial risk for direct sampling due to the steep gradients and loose substrate, and therefore sampling was conducted on nearby public properties. We selected K-12

schools as sampling locations to standardize sampling and to teach local schoolchildren in SE KY about the importance of pollinators. Pre-selection of sites involved locating all K-12 schools in each of the six SE KY counties and creating a 1000m buffer around each school using ArcMap 10.3. Next a layer including all surface mined areas in the six counties (Kentucky Mine Mapping Information Service, 2016) was added to the map. The map was visually scanned for areas where the mines did and did not overlap the 1000m buffer. Final site selection met the following two criteria (1) sites were separated by at least 2000m, and (2) an equal proportion of the sites were with and without surface mines within the 1000m buffer. Final permission for sampling on the school grounds was obtained for a total of eleven sites: seven schools containing a surface mine within a 1000m radius and four schools without any active or reclaimed mining operations within the 1000 m buffer.

Bee sampling and identification

The sampling location for each site included the entire schoolyard, and was always an area ≤ 500 m². Sampling occurred over three major periods: spring (March 15, 2017-May 31, 2017), summer (June 1, 2017-August 31, 2017), and fall (September 1, 2017-October 31, 2017). A combination of active and passive sampling methods were used to ensure adequate sampling of the bee community.

Passive sampling methods included trapping bees with UV blue, UV yellow, and white pan traps as well as blue and yellow vane traps. Pan traps were deployed for a 24- 48 hour period for up to three times per sampling season. Thirty pan traps were placed along a 150m transect at a distance of 5m apart alternating colors along the transect. The three colors used simulate UV pollen guides found on flower corollas and each color

attracts different portions of the bee community (Geroff et al., 2014). Pan traps were filled approximately 3⁄4 of the way full with a soapy water solution (Droege, 2008). Blue and yellow vane traps were hung from selected points (often fences or woody vegetation) in the sampling area. Vane traps were filled about ¾ of the way full with a soapy water solution (Droege, 2008) and were left out for 5-7 days unless inclement weather occurred. One trap of each color was deployed at least once per sampling period. Sweep netting was also performed at the time vane and pan traps were set, weather permitting. Net sampling has been shown to out-perform trap collecting in regards to species richness, but requires greater sampling effort (Popic et al. 2013).

It was our goal to perform at least one sample of each type once per sampling season at each site. However, this goal was only achieved for each site during the summer sampling period when schools were not participating in the data collection. Spring and fall samples were conducted in participation with teachers and students at the schools which led to some missing samples during these seasons. Additionally, one school participating in the experiment was permanently closed in May 2017, preventing a fall sample. The number of samples per season per site ranged from 0-10 in the Spring, 1- 3 in the Summer, and 0-4 in the Fall (Appendix A).

Bees returned to the lab for identification were first washed to remove debris such as pollen and plant material to aid in identification. Each sample was washed once with hot water and Dawn blue dish soap, shaken for 5 minutes, rinsed in hot water for 5 minutes, rinsed in cold water for 2 minutes, and briefly rinsed in 70% ethanol. Small bees with few hairs, such as *Ceratina spp.* and *Lasioglossum spp.*, were then removed from the bags and patted dry with a paper towel. Larger, hairier bees were placed in a covered

plastic hamster wheel and dried with an 1875-Watt hair dryer on high heat until all hairs were dry. Cleaned specimens were immediately pinned and labeled. All specimens were identified to genus or species level using morphological characteristics.

Landscape measurements

Percent land cover for each 1000m buffer was calculated using data from the 2011 National Landcover Database (NLCD 2011). The land cover classifications were simplified into the following 8 classes: herbaceous, forest, cropland, developed, barren, shrub, open water, and wetlands. The NLCD 2011 raster file was loaded into ArcMap 10.3 and then converted to polygons. Then a layer was created using the union function to combine the buffer layer and the NLCD 2011 polygons. The attribute table of this union layer was then exported and each land cover class was appropriately coded. The area of each land cover polygon was divided by the total area of the buffer to calculate the percent land cover of that polygon. Percent cover of surface mining was calculated using a similar method in addition to the 8 land cover classes described above. *Statistical analyses*

All data were analyzed using R version 3.3.3. A constrained correspondence analysis was used to understand which landscape variables were related to differences in bee community composition among sites. The following land cover variables were included as predictor variables in the initial full model: percent mine cover, mine presence/absence, percent forest cover, percent herbaceous cover, percent developed cover, percent shrubland, percent agricultural cover, and percent barren land cover. The two water-related land cover variables, percent cover of open water and wetlands, were not used in the analysis of bee community composition. The function CCA in the R

package vegan with Bray-Curtis distance was used to conduct the constrained correspondence analysis (Oksanen et al., 2018). Bee species abundances were quarter power transformed.

Results

Bee community composition

A total of 710 bees representing 100 species from 36 genera were collected from all other sampling methods combined. (Appendix A) summarizes the species collected from sites with and without surface mining in the landscape. Only two land cover variables were found to be significantly related to the variation in bee community composition among sites $(F_{2,8}=1.799; P=0.02; Figure 7)$. The presence/absence of surface mining in the landscape and the percent cover of herbaceous land together explained 31% of the variation in bee community composition; herbaceous cover and mine presence explained 17.29% and 13.72% of the variance respectively.

Bee species associated with surface mined landscapes included *Augochlora pura, Augochloropsis metallica, Ceratina strenua, Lasioglossum ms 15, Lasioglossum quebecense-ms2*. Bee species associated with a greater percent cover of herbaceous land

Figure 6. Canonical Correspondence Analysis plot displaying bee species driving landscape interactions. The CCA 1 axis displays the herbaceous cover interaction and the CCA 2 axis displays the interactions with the presence of a surface mine in the landscape.

in the landscape included *Anthophora abrupta, Halictus rubicundis, Lasioglossum*

cressonii, Lasioglossum zephyrum, and *Peponapis pruinosa*.

Discussion

Our results show that surface coal mining impacts the composition of the bee

community. Roughly one-fourth of the bee species collected from mined landscapes were

A. mellifera. The vegetative cover characteristic of reclaimed surface mines is dominated

by non-native grasses and forbs (Maigret et al. 2019), and higher numbers of generalist

bee species, such as *A. mellifera* are associated with lower quality or disturbed habitats

(Carman & Jenkins, 2016; Winfree et al., 2009).

The result that only two landscape variables measured in our study were significantly associated with variation in bee community composition among sites was surprising, considering the number of studies that have previously found other landscape variables to be associated with bee richness and abundance (Crist & Peters, 2014; Jha & Kremen, 2013; T. H. Ricketts et al., 2008; Tucker & Rehan, 2017). For example, other studies have concluded that higher proportions of forest cover tends to have an overall negative effect on species richness and overall bee abundance (Winfree et al., 2009, 2008; Winfree, Griswold, et al., 2007). Herbaceous land cover generally provides bees with floral diversity and appropriate forage and may be an important predictor of community composition (Bennett & Isaacs, 2014; Lowenstein et al., 2014; Svensson et al., 2000). Herbaceous cover generally has a positive overall effect on species richness and abundance (Bennett $\&$ Isaacs, 2014) and as such it is not surprising that herbaceous cover in the landscape was related to bee community composition. Several bee species can be considered drivers for this interaction based on their ordination position. These species include *Anthophora abrupta, Halictus rubicundis, Lasioglossum cressonii, Lasioglossum zephyrum,* and *Peponapis pruinosa.* All of these species are ground nesting bees, and most are generalists or have little foraging information available (Blitzer, Gibbs, Park, & Danforth, 2016; Graham et al., 2015; Roulston & Cane, 2002; Soucy, 2006; Ullmann, Meisner, & Williams, 2016). Two of the species, *Halictus rubicundis* and *Lasioglossum zephyrum* are primitively social, while the rest are solitary (Roulston & Cane, 2002; Soucy, 2006). While all of these bees are ground nesting, a variety of soil preferences are represented. *Anthophora abrupta* builds gregarious nests in hardened clay soils(Graham et al., 2015), while *Halictus rubicundis* prefers sandy loam soils. One

specialist is represented among these species, *Peponapis pruinosa,* which forages exclusively on plants in the family Cucurbitaceae

The presence/absence of surface mining in a landscape constrained the second ordination axis and was the only other significant landscape predictor variable associated with bee community composition. Three families were represented among species highly correlated with mine presence; Apidae, Halictidae, and Megachilidae. The species of Apidae were *Apis mellifera, Bombus impatiens, Bombus bimaculatus,* and *Bombus pennsylvanicus.* All of these species are social and dietary generalists. Species in the Halictidae included: *Augochlora pura, Augochlorella aurata, Augochlorella persimillis, Lasioglossum foveolatum,* and *Lasioglossum smilacinae.* Little detailed information is available on these 2 species of *Lasioglossum*, but they are presumed to nest in the ground and be dietary generalists (Onuferki, Kutby, & Richards, 2003; Packer, Genaro, & Sheffield, 2007). Species in *Augochlora* and *Augochlorella* are dietary generalists, but have different nesting habits (Ordway, 1966; Packer et al., 2007; Stockhammer, 1966). *Augochlorella pura* nests in dead or rotting wood, while species in *Augochlorella* nest in the soil (Ordway, 1966; Packer et al., 2007; Stockhammer, 1966). One representative of Megachilidae was associated with mined land, *Megachile petulans* for which no conclusive natural history information could be found.

Though availability to floral resources generally acts as the primary determinant of the status of a bee community, there is evidence that suggests that nesting habitats and resources may also play a key role in bee abundance and richness (Grundel et al., 2010; Potts et al., 2005). The availability of ideal nesting habitats could have also affected our results. Reclaimed mine sites might not provide some bee species appropriate nesting

habitat, therefore, they are not found at these locations. For instance, Potts et al. (2005) found that the availability of bare ground and nesting cavities were the two key factors influencing the structure of entire bee communities. Some species also preferred steep and sloping ground, abundance of plant species with pithy stems, and pre-existing burrows (Cane, 1991; Potts et al., 2005). The landscape of a reclaimed surface mine can be described as grassland or forested area, the purpose of reclamation is to return the land used for mining back to its near original state. The reclamation approach mandates a suitable rooting medium for tree growth comprised of topsoil from backfill, weathered sandstone, and/or the best available medium, non-compacted soil growth medium, reconstructed water-ways, and high quality and hardy tree and plant species (Davis 2006), to name few, which in turn may be unattractive and uncharacteristic to some bee species.

Our results demonstrate the community wide impact of surface mining on bees at the landscape scale. Conservation efforts on surface mines should still be considered, despite the comparable abundance between mined and unmined sites, in the reclamation and restoration process to provide habitat for rare and endangered bee species. Kentucky was once part of the range of the now endangered rusty-patched bumblebee, *Bombus affinis* and the declining yellow-banded bumble bee, *Bombus terricola* (S. R. Colla & Packer, 2008; Evans, Thorp, & Jepsen, 2008)*.* Creation of appropriate habitat on reclaimed surface mines could provide an environment in which these bumblebees could be re-established through re-release and careful management. For example, reclamation of surface mines could include floral mixes with a specific floral composition that promotes desirable pollinator communities or provides floral resources for bee species

with declining population numbers. Planting larger plots of wildflowers with a more diverse combination of flower species are more suitable for the conservation of wild pollinators but also contribute to protecting native generalist bee species (Blaauw & Isaacs, 2014).

Our results also suggest that the current practices for reclamation provide suitable habitat for honey bees and bumblebee foragers. Constructing large apiaries on these otherwise unused lands can be used to improve, rebound, and stabilize disturbed landscapes, boost economy for commercial beekeepers, and restore honey bee populations. Improvement upon these practices focused on the conservation of a broader assemblage of native pollinators could include the addition of other artificial nesting habitats on reclamation sites to encourage nesting of other bee species. Nesting options might create ideal habitats for some bees, thus attracting more bees to these sites. Encouraging both domestic and wild/native pollinators to reclaimed surface mine sites would further promote not only stability in the plant-pollinator network but also the bee assemblage as a whole.

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APPENDICES

Appendix A: Species richness and abundances by site. The sites are as follows: A-A.B. Combs Elementary; B- Arlie Boggs Elementary; C- Beaver Creek Elementary; D- Emmalena Elementary; E- Fleming-Neon Middle School; F- Hazard High School; G- Leatherwood Elementary; H- London Elementary; I- Martha Jane Potter Elementary; J- Mountain View Elementary; K- Paces Creek Elementary.

Appendix A: Species richness and abundances by site. The sites are as follows: A-A.B. Combs Elementary; B- Arlie Boggs Elementary; C- Beaver Creek Elementary; D- Emmalena Elementary; E- Fleming-Neon Middle School; F- Hazard High School; G- Leatherwood Elementary; H- London Elementary; I- Martha Jane Potter Elementary; J- Mountain View Elementary; K- Paces Creek Elementary.

Appendix A (continued)

Appendix A (continued)

Аррепиіх А (conunuea)												
	Site											
Species	A	B		D	Ε	F	G	Н		J	K	Total
kenoyeri												
lignaria												
pumila								10				
simillima												$\overline{2}$
taurus												ി
texana												
Peponapis	$\overline{4}$		$\overline{2}$	3								
pruinosa	4		\mathcal{D}	3								
Sphecodes												
smilacinae												
Xylocopa												ി
virginica												2
Total	85	70	13	69	18	58	50	265	41	23	18	710

Appendix A (continued)

Appendix B: Bee species richness and abundance for mined and unmined sites.

Bee Species	Unmined	Mined	Total
Agapostemon	14		14
texanus	$\mathbf{1}$		1
virescens	13		13
Andrena	37	$\overline{2}$	39
<i>barbilabris</i>	$\mathbf{1}$		$\mathbf{1}$
carlini	8		8
cressonii	5	$\mathbf{1}$	6
dunningi	6		6
erigeniae	\overline{c}		\overline{c}
geranii	$\mathbf{1}$		$\mathbf{1}$
nasonii	3		3
phaceliae	5		5
pruni	$\mathbf{1}$		$\mathbf{1}$
sayi	$\mathbf{1}$		$\mathbf{1}$
violae	$\overline{2}$		$\overline{2}$
wheeleri		$\mathbf{1}$	$\mathbf{1}$
ziziaeformis	$\overline{2}$		\overline{c}
Anthidiellum		$\mathbf{1}$	$\mathbf{1}$
notatum		1	1
Anthidium	3		3
manicatum	$\mathbf{1}$		$\mathbf{1}$
oblongatum	$\overline{2}$		\overline{c}
Anthophora	$\overline{2}$	$\mathbf{1}$	3
abrupta	$\mathbf{1}$	1	$\overline{2}$
bomboides	$\mathbf{1}$		$\mathbf{1}$
Apis	34	37	71
mellifera	34	37	71
Augochlora	6	\overline{c}	8
pura	6	$\overline{2}$	8
Augochlorella	60	22	82
aurata	56	17	73
persimillis	$\overline{4}$	5	9
Augochloropsis		1	$\mathbf{1}$
metallica		$\mathbf{1}$	1
Bombus	23	25	48
bimaculatus	8	11	19
griseocollis	3	$\mathbf{1}$	$\overline{4}$
impatiens	5	12	17

Appendix B: Bee species richness and abundance for mined and unmined sites.

