Land use in the Northern Great Plains region of the U.S. influences the survival and productivity of honey bee colonies

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Abstract

The Northern Great Plains region of the US annually hosts a large portion of commercially managed U.S. honey bee colonies each summer. Changing land use patterns over the last several decades have contributed to declines in the availability of bee forage across the region, and the future sustainability of the region to support honey bee colonies is unclear. We examined the influence of varying land use on the survivorship and productivity of honey bee colonies located in six apiaries within the Northern Great Plains state of North Dakota, an area of intensive agriculture and high density of beekeeping operations. Land use surrounding the apiaries was quantified over three years, 2010-2012, and survival and productivity of honey bee colonies were determined in response to the amount of bee forage land within a 3.2-km radius of each apiary. The area of uncultivated forage land (including pasture, USDA conservation program fields, fallow land, flowering woody plants, grassland, hay land, and roadside ditches) exerted a positive impact on annual apiary survival and honey production. Taxonomic diversity of bee-collected pollen and pesticide residues contained therein varied seasonally among apiaries, but overall were not correlated to large-scale land use patterns or survival and honey production. The predominant flowering plants utilized by honey bee colonies for pollen were volunteer species present in unmanaged (for honey bees), and often ephemeral, lands; thus placing honey bee colonies in a precarious situation for acquiring forage and nutrients over the entire growing season. We discuss the implications for land management, conservation, and beekeeper site selection in the Northern Great Plains to adequately support honey bee colonies and insure long term security for pollinator-dependent crops across the entire country.

Keywords agriculture, land use, *Apis mellifera*, colony survival, honey bee, honey production, pesticide exposure, pollen collection

1. Introduction

The phenomenon of sustained and elevated annual losses of honey bee colonies continues to severely impact the US beekeeping industry (Steinhauer et al. 2014; Lee et al. 2015). Such losses have been mainly confined to North America and parts of Europe (NRC 2007; vanEngelsdorp et al. 2008; Potts et al. 2010), and specifically, annual losses for commercial beekeepers in the US have hovered around 30% since 2006-07, with a low of 22% in 2011-12 and a high of 40% in 2012-13 (vanEnglesdorp et al. 2007, 2008, 2010, 2011, 2012; Spleen et al. 2013; Steinhauer et al. 2014; Lee et al. 2015). Numerous pests, diseases, and pesticides have been implicated in potentiating colony failure, both alone and in combination (Cox-Foster et al. 2007; vanEngelsdorp et al. 2009; vanEngelsdorp et al. 2013).

Because of these continued, and seemingly ubiquitous annual losses, more attention has turned toward how landscapes and land use influence factors related to colony health that may ultimately differentially impact the productivity and survival of honey bee colonies. For example, pollen is primarily required to raise brood and contribute to sustained colony population growth throughout the growing season, but critically, protein nutrition also moderates the impacts of honey bee pathogens, parasites, overall resistance and resilience to stress factors, and foraging behavior (Alaux et al. 2011; Huang 2012; Scofield and Mattila 2015). High quality and abundant pollen contributes to increased nutritional stores and an overall decreased (quieter) immune status in individual bees (Alaux et al. 2010; Smart et al. 2016). Further, honey bees maintained on a high quality pollen diet exhibit increased longevity when infected with a fungal parasite (Di Pasquale et al. 2013), and honey bees exhibit lower viral levels when maintained on pollen versus sugar syrup or pollen substitute (DeGrandi-Hoffman et al. 2010). The potential impacts of land use via differential nutrition are wide-ranging, including the effects of adequate and sustained floral resource availability and diversity and interactions with environmental pesticide exposure which may influence the nutrition, immune systems, and survival of honey bee colonies (e.g. Naug 2009; Pettis et al. 2013; Smart et al. 2016).

The Northern Great Plains (NGP) region, including North Dakota, South Dakota, Montana, and Minnesota, has acted as an unofficial "bee refuge" for a large proportion of the managed, commercial honey bee colonies throughout the growing season. Colonies transported to this area of the country for the summer by migratory beekeepers have done well due, in large part, to the presence of an abundance of nectar and pollen-producing flowers. Historically, this region has had less extensive monocultural agriculture compared to regions farther south (e.g. the Midwestern corn belt). This region hosts around 1 million honey bee colonies from May-October every year, representing approximately 40% of the total US managed, commercial pool of honey bee colonies (USDA 2014b). Critical regional blooms include perennial clovers and alfalfa, canola, sunflowers, wildflowers, and, more broadly, contributions from volunteer plant species located in certain land use types such as livestock-grazed pastures and grasslands. Other important types of land use containing forbs are USDA conservation program fields, such as the Conservation Reserve Program (CRP), which is a government program incentivizing landowners to set aside highly-erodible and other sensitive lands into long term conservation covers (Gallant, Euliss and Browning 2014).

In recent years, increasing numbers of colonies have been transported to California to pollinate a single crop, almonds. The approximately 1 million bearing acres of almonds in CA are 100% dependent on the pollination that they receive from honey bees. Currently, approximately 1.5 million of the 2.5 million available colonies nationwide undertake the journey to the central valleys (San Joaquin and Sacramento) of California, many originating from the NGP.

Surprisingly, implications of land use on resource quality, honey bee health, and survival have been considered in relatively few (and recent) studies (e.g. Naug 2009; Odoux et al. 2012; Clermont et al. 2015; Requier et al. 2015; Smart et al. 2016). Other research has focused on spatial foraging patterns of honey bee colonies, and distances of various crops and land use features relative to colony position (e.g., Beekman and Ratnieks 2000; Steffan-Dewenter and Kuhn 2003; Couvillon, Schurch and Ratnieks 2014). Recent studies tracking survival of colonies in US migratory beekeeping operations (e.g. Runckel et al., 2011; vanEngelsdorp et al., 2013) did not quantify the health and survival of colonies in relation to specific landscape patterns or features to which the colonies were exposed.

The overarching objective of this study was to quantify the relationship between land use composition and honey bee productivity and survival in the Northern Great Plains region of the US. We followed colonies positioned in six apiaries over three years and hypothesized that survival and honey production would be higher for apiary sites surrounded by a greater amount of land use in potential bee forage (uncultivated forage land, cultivated forage land, and wetlands, Fig. 1) due to a greater presence of nectar and pollen-producing forbs and woody plants in those areas of the landscape. Row crops did not dominate such areas and thus colonies were predicted to experience a greater abundance and diversity of floral resources and overall reduced exposure to agricultural pesticides. Our specific objectives were to 1) identify land use within the larger agricultural matrix associated with higher colony survival and productivity among apiary sites, 2) build a predictive statistical model relating land use to survival and honey production of apiaries, and 3) identify taxonomic origin of bee-collected pollen, identify pesticide residues within the pollen, and describe and compare overall pollen diversity among study sites against the backdrop of varying land use.

2. Materials and methods

2.1 Land use assessments

For each of three years (2010-2012), land use in North Dakota was extensively surveyed on the ground within a 3.2-km (2-mile) radius around each of six sites (apiaries) (Fig. A.1). We chose this scale as a realistic total area (approx. 32 km^2) over which bee colonies at a given site would be expected to forage (Visscher and Seeley 1982; Beekman and Ratnieks 2000). We also analyzed more localized foraging radii (500m, 1000m, and 2000m). The average distance between sites was 40 km (9-68 km range). Broad land use categories included: CRP, ditch, fallow land, flowering woody plants and shrubs, grassland, hay land, pasture, alfalfa, canola, sunflower, wetlands, corn, oats, soybeans, and wheat (Table A.1). These broad land categories were subsequently combined into five groups for statistical analyses, including: 1) Uncultivated forage land (CRP, ditch, fallow, flowering woody plants, grassland, hay land, pasture); 2) Cultivated forage land (alfalfa, canola, sunflower); 3) Wetlands; and 4) Non-forage (corn, oats, soybeans, wheat). Sites were lettered (A-F) in descending order of land area in uncultivated and cultivated forage land, i.e. a gradient from high to low expected usefulness to honey bees (Fig. 1).

A surveyor visited each site three times (once each spring in May-June, summer in July-early August, and autumn in late August-September) each year to verify land use in the field and this data, in addition to data from the National Agricultural Statistics Survey (NASS), were entered into ArcGIS v.10 for final quantifications of the area of various types of land use within the 3.2-km radius around each site. Additionally, during each visit the surveyor visually assessed and estimated floral cover of the most commonly occurring flowers within each land category around each site including, sweet clover *Melilotus* spp.; alfalfa *Medicago sativa*; gumweed *Grindelia squarrosa*; native sunflower *Helianthus* spp.; sow thistle *Sonchus* spp.; and goldenrod *Solidago* spp.). The percent floral cover estimates were then converted to a summed total area of each species occurring within the 3.2-km around each site over three years (Table 1).

Proprietary CRP data was accessed via an FSA/USGS Interagency Agreement. One site, Site B, was located inside the Arrowwood National Wildlife Refuge; approximately 75,000 acres of U.S. Fish and Wildlife Service (FWS) land composed primarily of grassland. A special use permit was granted to allow honey bee colonies access to this site. Colonies positioned in this area had access to FWS lands to the west and north but were adjacent to agriculturally managed private lands to the east, outside the refuge.

2.2 Colony health monitoring

Initiation of colonies occurred each spring (May), comprised of a freshly mated *Apis mellifera ligustica* queen and approximately 10,000 workers per colony. Honey bee colonies owned and managed by a local commercial beekeeper were positioned among the six apiaries in North Dakota from 2010 through 2013 (common apiary size for this beekeeper is 48 colonies per site; we assessed 24 (half) for survival and honey production). Colonies were maintained in a typical US commercial beekeeping configuration consisting of four colonies per pallet and 12 pallets per apiary, facilitating movement of colonies into and out of the apiary via forklift. Each colony was tagged with a unique number for identification. Colonies remained in North Dakota from May-September each year. In autumn (October), colonies were loaded onto trucks and shipped to California where the colonies were temporarily placed in holding yards (until moved into almonds). Starting in mid-February, the colonies were transported from holding yards into almond orchards for pollination. Colonies that died each year were replaced by the beekeeper with new colonies (and queens) before they returned to North Dakota each May.

Colony health was monitored in each of the 24 colonies per site every 6 weeks year-round for a variety of health metrics (Smart et al. 2016). *Varroa destructor* mites and *Nosema* spp. were controlled in all colonies according to the beekeeper's management regimes and overall infestation levels were low (Smart et al. 2016). Honey production was determined by weight of honey boxes removed from each colony and calculated as the annual average weight (kg) per site.

Annual apiary survival was determined as the number of surviving colonies out of 24 per apiary from May of each year (in North Dakota) through March of the following year (almond bloom in California). March was chosen as the cut-off point for survival because this was when the beekeeper made a decision as to which colonies were suitable to be moved into almonds to fulfill pollination contracts; culling dead colonies in the process. Additionally, 90% of colonies that survived to almond pollination were alive and healthy by the end of the almond bloom.

2.3 Collection and taxonomic identification of pollen

Three colonies were maintained at each of the six locations in North Dakota for pollen collection each year; these colonies were not included in the regularly assessed 24 colonies. These colonies were fitted with pollen traps that, when opened, forced returning foraging bees to walk through screens upon entering the hive, which dislodged pollen loads from the hind tibiae into a pollen collection drawer. Traps were open for a 24-hour period 3-6 times per summer (six in 2010, five in 2011, three in 2012), and pollen was collected into a plastic bag and placed in a cooler containing dry ice for shipping. There was no pollen recovered on certain sample dates and sites. Upon arrival at the USDA-ARS-Bee Research Lab in Beltsville, Maryland, samples were stored at -20°C until analyzed. A randomly chosen, mixed 3-gram pollen subsample from each site and date was sorted first by color to narrow down taxonomic diversity within a sample and then the proportional make-up of each color was subsequently identified to taxonomic plant of origin using light microscopy. The proportion of each taxon in the total 3g mixed sample from a given apiary and date was then back calculated to arrive at the proportion of each taxa from each specific apiary and date. The pollen diversity index was calculated based on all taxa detected in each year, 2010-2012. Attempts were made to identify pollen to the lowest taxonomic level possible, though in many cases certain pollens could only be identified to genus or family, or remained 'undetermined' (Table A.2).

2.4 Pesticide residue analysis of pollen samples

An additional separate 3-gram subsample of fresh pollen from each site and date was sent to USDA-AMS-National Science Laboratory in Gastonia, NC for pesticide residue analysis. Results were reported in parts per billion (ppb) for 174 commonly used insecticides, fungicides, herbicides and metabolites. The amount of each residue in ppb detected from May through September was averaged from each site, and was used to calculate a pollen hazard quotient (HQ), defined as the ppb of a given pesticide divided by its contact LD_{50} (Stoner and Eitzer 2013). Hazard quotients were averaged annually to analyze their relationship with land use, survival, and honey production among apiary sites. Contact LD_{50} values may be a conservative estimate of exposure because they are often less toxic (higher LD_{50}) compared to oral LD_{50} values for the same pesticide (Stoner and Eitzer 2013; Sanchez-Bayo and Goka 2014). Contact LD_{50} values used for calculating HQ were determined by averaging reported values from 4 sources (Mullin et al. 2010; Stoner and Eitzer 2013; Sanchez-Bayo and Goka 2014; and the EPA Office of Pesticide

Programs Ecotoxicity Database USEPA 2014). Importantly, pollen hazard quotients fail to account for synergistic or inhibitory interactions between and among pesticides. However the HQs do allow for a comparison of the relative overall pesticide exposure among sites in a more biologically relevant manner compared to strictly summing and comparing ppb, which does not take into account the variable toxicities of different chemicals.

2.5 Statistical analysis

Statistical analyses were carried out using R version 3.1.1 (R core team, 2014-07-10). For objective 1, simple linear regression and ANOVA analyses of land use data by site and year were first conducted to evaluate the effects of land use on survival and honey production. For objective 2, data were then analyzed using lme4 (Bates, Maechler and Bolker 2014) linear mixed effects modeling to examine the relationship between the predictor (area of bee forage land (log-transformed m^2)) and two main responses: 1) annual apiary survival (number of colonies surviving out of 24 at each site and year); and 2) apiary honey production (mean kg per year). Site and year were specified as random effects. Akaikae's Information Criterion corrected for small sample size (AICc) was used to rank the multiple competing models of land use on survival or honey production. We calculated AICc weights (w) and evaluated 95% confidence intervals to determine the relative importance of model parameters. Finally, diversity (objective 3) was analyzed via determination of the Shannon-Weiner Diversity Index (land use and pollen taxonomy) by site using the vegan package 2.2.1 in R and Pearson correlation analyses were conducted relating pollen diversity and pesticide HQ to land use, survival and honey production.

3. Results

3.1 Objective 1: Relationships among land use, honey production, and colony survival

There were differences in the type of land use (m^2) within the 3.2-km area across the land use gradient (Fig. 1). In the uncultivated forage land category, the availability of floral resources varied widely (Table 1). For example, despite similar total areas of land in CRP over the three years near sites A and F (summed total of approximately 9 million m^2), the estimated total floral cover was vastly different (84% and 20%, respectively). The land use categories shown in Table 1 contained the majority of floral resources (and other taxa not listed in Table 1) as determined by on-the-ground surveys within the 3.2-km radius of each site, and thus represent the most likely targets for honey bee foraging.

There was a strong positive linear relationship between the area of uncultivated forage land surrounding an apiary and annual apiary survival ($F_{1,16}$=15.69, r^2=0.50, p=0.001, Fig. 2a). Similarly, there was a positive, though not statistically significant, relationship between the amount of uncultivated forage land and honey production and ($F_{1,16}$=2.14, r^2=0.12, p=0.16, Fig. 2b). Annual survival and honey production were significantly positively related ($F_{1,16}$=12.11, r^2=0.43, p=0.003, Fig 2c). This relationship was primarily driven by the low survival and productivity of colonies at site F.

ANOVA of survival indicated a significant impact of site (i.e. varying land use across a gradient) on the number of colonies surviving each year ($F_{5,12}$=6.6, p=0.003), with significantly more colonies surviving at sites A and D compared to site F (Fig. 2d). ANOVA for honey production (Fig. 2e) indicated that site was not a significant contributor ($F_{5,10}$=1.73, p=0.22) but year did have a significant effect ($F_{2,10}$=5.71, p=0.02) wherein honey production in 2011 was lower compared to 2012, but not different from 2010.

Because sites A and F represented the extremes of apiary survival, we investigated the impact of removing the data points from those two sites. Removal of all data from either site alone still resulted in statistically significant linear models (Remove site A: $F_{1,13}$=6.30, r^2=0.33, p=0.03; Remove site F: $F_{1,13}$=6.18, r^2=0.32, p=0.03), while removing both sites resulted in a non-significant relationship ($F_{1,10}$=0.31, r^2=0.03, p=0.59) between uncultivated forage land and survival.

3.2 Objective 2: Linear mixed modeling of land use on survival and honey production

Linear mixed effect modeling indicated that the area of uncultivated forage land was the best statistical predictor of apiary survival (Table 2), better describing the variation in survival than cultivated forage land, wetlands, or any additive combination of predictor variables. Examination of the evidence ratios for the best models of survival indicated the model including only uncultivated forage land was greater than 6 times more predictive of colony survival than the model with wetlands added (Evidence ratio (*E)* = 0.729/0.117), and approximately 7.5 times more predictive than the model including cultivated forage land (*E* = 0.729/0.096). The 95% confidence intervals for wetlands and cultivated forage land coefficients overlapped zero (Table 2), further indicating that the presence of uncultivated forage land was the main land use driver of apiary survival. The area of wetlands varied little among sites, but surprisingly had an overall negative effect on survival and honey production.

Similarly, total area of uncultivated forage land best predicted honey production (Table 2) however, other competing models including wetlands and cultivated forage land areas could not be ruled out (i.e. < 2 ΔAICc, low evidence ratios, Table 2). While the dependence of uncultivated forage land area on an apiary's survival was well supported by our data, the dependence of uncultivated forage land for honey production was only weekly supported compared to other models that included wetlands and cultivated forage land area.

We also investigated the impact of land use on survival and honey production at more localized spatial scales (Table A.3). At decreased spatial scales (500m, 1000m, 2000m radii) the area of cultivated forage land continued to be the land use feature most predictive of apiary survival, though our 3.2-km radius models maintained lower AICc and values greater weights comparatively. For honey production at more localized spatial scales, cultivated forage land (alfalfa, canola, sunflower) emerged as the most indicative land use feature, compared to cultivated forage land at the 3.2-km radius (Table A.3).

3.3 Objective 3. Pollen: identification and pesticide residue analysis

A total of 18 different plant families including 33 genera (Fig. 3a) were detected from pollen traps over the three years of the study. Three families (Asteraceae, Brassicaceae, and Fabaceae) together made up the majority of bee-collected pollen in these landscapes, providing up to 57%, 26%, and 81%, respectively (39-94% overall) of the total pollen collected over the three years. Cultivated plant genera including alfalfa (*Medicago*), field bean (*Phaseolus*), canola (certain *Brassica*), sunflower (certain *Helianthus*), and soybean (*Glycine*) made up relatively little of the total collected pollen (Fig. 3a), site A: 17%, site B: 12%, site C: 8%, site D: 10%, site E: 8%, site F: 3%). Soybean pollen specifically, though detected, was relatively rare, occurring only at site B (0.4% in 2010), and site F (2% in 2010). No corn pollen was detected in any samples in any year.

Fabaceae and Brassicaceae pollen were represented in the late spring through mid-summer, while Asteraceae became more predominant mid-summer through early autumn (Fig. 3a). One genus of Fabaceae, *Melilotus* spp., was particularly persistent in bloom time (pollen present in samples from late June through early September) and dominant in proportion of the total pollen collected by the bees (Fig 3a, site A: 2-39%, site B: 13-66%, site C: 7-47%, site D: 2-29%, site E: 9-45%, site F: 18-35%) over the three years. In fact, many of the most commonly collected genera/species of plants identified in this study were non-native to the U.S., including *Centaurea* spp., *Cichorium* spp., *Circium* spp., *Medicago sativa* (cultivated), *Melilotus* spp., *Silene latifolia*, *Sonchus* spp., *Taraxacum officinale*, and *Tragopogon* spp. Several native species, and other potential natives depending on the species within the genera identified, were also found including *Grindelia squarrosa, Helianthus* spp. (cultivated or wild), *Lathyrus* spp., *Lupinus* spp., *Phaseolus* spp. (cultivated), *Solidago* spp., *Trifolium* spp., and *Vicia* spp. (Fig. 3a).

The Shannon-Weiner diversity index of large-scale land use (3.2-km radius) showed that the highest diversity was present around sites A-D (Fig. 4). Bee-collected pollen from sites A and F exhibited the highest, and sites B-E the lowest, annualized taxonomic diversity (Fig. 4). The diversity of bee-collected pollen was not correlated with annual survival ($t= -0.59$, $df=16$, $r= -0.15$, $p=0.56$, 95% CI: -0.57, 0.34), or honey production ($t= -0.29$, $df=16$, $r= -0.07$, $p=0.78$, 95% CI: -0.52, 0.41). Additionally, no significant statistical relationships were found between pollen diversity and land use diversity or the amount of uncultivated forage land, i.e. greater land use diversity or amount of uncultivated forage land surrounding an apiary did not equate to greater diversity of collected pollen, and further, this lack of a relationship was conserved when examined at more localized spatial scales.

Pesticide residues from agricultural and beekeeper applications were detected in the fresh pollen collected throughout the growing season among all sites and years (Fig. 3b, Table A.4). Although colonies were exposed to a number of pesticides over the three years, no statistically significant impacts of pesticide exposure on colony survivorship or honey production were found (impact of pollen pesticide hazard quotient on survival: $F_{1,16}$=0.75, p=0.40, and honey production: $F_{1,16}$=0.03, p=0.86) and, further, we did not find any correlative relationship between total annual pollen pesticide residue and the area of land use surrounding apiaries in non-forage crops (t= -0.25, df=16, r= -0.06, p=0.81) or land diversity (t=0.004, df=16, r=0.001, p=0.99) . This pattern held when considering land use at more localized spatial scales (500m, 1000m, 2000m radius from apiaries). In terms of overall hazard quotient, sites A and E had the highest, while sites B, D, and F had reduced HQ (Fig. 3b). However, nearly 80% of the elevated HQ determined at site A was due to a single detection of deltamethrin (Fig. 3b). Generally, the most toxic agricultural chemicals that were found (e.g. bifenthrin, chlorpyrifos, cyhalothrin, deltamethrin) occurred in the latter portion of the summer, presumably used as sprays for managing crop pest populations that built up over the season.

Notably, no neonicotinoid insecticides were detected in pollen at any sites over the three years. Nine insecticides with high toxicity to bees were detected, two organophosphates (OPs), six pyrethroids, and one partial systemic (Table A.4). Of the two OPs, chlorpyrifos was most commonly found, detected in pollen from all sites throughout the season (Fig. 3b). Of the seven pyrethroids detected (six of which have high toxicity to honey bees), cyhalothrin was most commonly found variably from all sites. Four of the other pyrethroids: bifenthrin, cyfluthrin, cypermethrin, and esfenvalerate were found sporadically across the sites and years. Deltamethrin was detected only once at site A on 08/17/2010.

In addition to the aforementioned insecticides, agriculturally-applied fungicides and herbicides were also detected. Overall, five fungicides (all with low honey bee toxicity) were detected but the most commonly found fungicide was carbendazim. Chlorthanlonil has low toxicity to honey bees, and was detected in the early season at all sites except F (Fig. 3b). The other four fungicides: pyraclostrobin, tebuconazole, and vinclozolin were each only detected on one sample date and site each. Finally, four detections of three herbicides were found: oxyfluorfen, pendimethalin, and trifluralin.

Residues of six beekeeper-utilized pesticides (and metabolites) were among the most commonly detected chemicals across all sites and years and included coumaphos, coumaphos oxon, fluvalinate, fenpyroximate, thymol, and 2,4 Dimethylphenyl formamide (DMPF) a breakdown product of the miticide, Amitraz. Paradichlorobenzene, a chemical used as a fumigant to deter stored beekeeping equipment pests, such as wax moths, was detected at all sites only in 2011. The toxicities of thymol, DMPF, and paradichlorobenzene are not known. The other detected products have low or moderate toxicity to bees (Table A.4).

4. Discussion

This study demonstrated the influence of land use on the survival and honey production of colonies in a US commercial beekeeping operation. We found strong support for the amount of uncultivated forage land during the summer on the ultimate survival of colonies over the winter. Importantly, we previously showed that pests, parasites, and diseases did not vary among the six apiaries (Smart et al. 2016) and here, we observed a lack of significant differences in overall pesticide exposure among apiaries related to land use and survival. Therefore, we provide strong quantitative evidence that land use alone significantly impacts the annual survival of commercial honey bee colonies in the NGP.

The 12-17% annual mortality over the three years at site A fell within the “acceptable range” of beekeeper expected losses (Steinhauer et al. 2014), and was much closer to annual losses prior to the establishment of the *V. destructor* mite to the US in the 1980s (D. vanEngelsdorp, pers. comm.). Site A also possessed the greatest area of uncultivated forage land (approx. 70%) in the surrounding land over the three study years. Conversely, the 50% annual mortality at site F was well above the national average of around 30% (Lee et al. 2015), and this site was the least diverse in overall land use, and further, possessed the least amount of uncultivated forage land (around 10% of the total area), most of which was not florally productive.

4.1 Pollen and land use diversity

Our previous work suggested that the quantity of pollen collected, brood quantity, *Varroa* mite levels, and physiological measures of nutrition and immunity were significant metrics of annual colony survival (Smart et al. 2016). The quantity of pollen, rather than the diversity of pollen, collected among apiaries was more related to survival, which we show here, is a function of land use. The amount of pollen collected, related to the abundance of pollen available in the landscape, may be more critical for generalist-foraging honey bee colonies than highly diverse floral resources. However, because we averaged pollen diversity annually we caution that diversity of pollen may be critical at particular times of the season. Further, honey bees located in landscapes not dominated by intensive mono-cultural agriculture like those in our study region may display different foraging patterns relative to the availability of floral resources in the surrounding landscape.

Site A, with moderate land use diversity at the 3.2-km radius, was comprised of land where a lot of flowers *could* grow (e.g. CRP, grassland, hayland, pasture), and was relatively abundant in commonly occurring floral resources in those areas. This contributed to moderate to high overall pollen taxonomic diversity and greater total pollen collection at site A (Smart et al. 2016). Additionally, a large component of the uncultivated forage land surrounding site A was pasture, where volunteer species utilized by honey bees were commonly found growing in abundance. In contrast, sites E and F had moderate to low land use diversity and the types of land use where flowers *could* grow en masse (e.g. CRP, grassland, hayland) were relatively absent or devoid of floral coverage. Sites E and F also had a large proportion of flowers in ditches (a landscape feature that is widely distributed and ephemeral due to mowing and spraying regimes). Interestingly, honey bee colonies lowest on the gradient (site F), along with site A, collected a relatively high diversity of pollen, both at the family and genus levels.

Characteristics of low gradient sites, such as smaller flower patches or widely distributed resources like those in roadside ditches, require more time to trigger recruitment (Dornhaus and Chittka 2004; Beekman and Lew 2008). As a result, foragers in landscapes characterized by such features may actively search for, and come into contact with, a greater overall diversity of flowers. An optimal foraging pattern could partially explain the trends we observed given the overall availability of floral resources near our apiaries, wherein colonies increased diet breadth in low resource landscapes and decreased diet breadth in relatively higher resource landscapes (Kunin and Iwasa 1996; Fontaine, Collin and Dajoz 2008). Site F, specifically, had a large amount of conservation (CRP) land nearby that may have provided the colonies with a greater diversity of floral resources compared to other low gradient sites without appreciable conservation lands nearby.

Pollen from one plant genus, *Melilotus* spp., was identified in all years and sites (except site E in 2012), highlighting the relative preference for this copious nectar- and pollen-producing biennial volunteer plant. Experimental colonies fed *Melilotus* spp. pollen have been shown to produce more brood compared to several other single source and blends of pollen, and sweet clover was most preferred by the bees (Campana and Moeller 1977). Aside from *Melilotus* spp., most of the other plants from which pollen was collected were those that were not actively cultivated, as has been reported in other cropping systems (Pettis et al. 2013; Requier et al. 2015). In addition to pollen resources, many of these plants are also abundant nectar sources for honey production, including the genus, *Melilotus*. In the current study, cultivated bee forage plants (sunflower, alfalfa, canola, beans) comprised, on average, only 10% of the total pollen collected across all sites and years, and further, occurred as relatively brief, punctuated mass blooms over the summer. The lack of cultivated flowering plants puts into perspective the heavy reliance of honey bee colonies on volunteer, and often non-native, flowering resources in these highly bee-populated agricultural lands that are susceptible to loss through herbicide use, mowing and degradation over time.

We chose a 3.2-km radius around each site as a reasonable foraging range for honey bee colonies. This radius encompassed approximately 32-km^2 of surrounding agricultural land. We also considered relationships between land use and survival, and land use and honey production at more localized spatial scales and found that in both cases, the relationship was most significant at the largest scale (3.2-km radius). Interestingly, despite a minimal amount of cultivated forage crop land (e.g. alfalfa, canola, sunflower) near our study apiaries, we found that such crops were important for honey production at smaller, localized scales. Given honey bees forage over a potentially vast area, future work should consider the appropriate spatial scale at which land use most exerts its influence on the health, productivity, and survival of honey bees colonies. Such an understanding would assist beekeepers, policy makers and land managers in gaining the most reward out of the limited amount of land available for pollinator forage and habitat enhancement efforts.

4.2 Pesticide exposure

The relatively high diversity of pollen collected within and among apiaries, coupled with the presence of unidentified pollen on every date, made it impossible to associate certain pollen taxa with pesticide exposure. However, the general lack of agricultural crop-derived pollen indicated that pesticide drift from target fields during or after application onto flowers growing in surrounding areas was the most likely route for such agricultural pesticide exposure by honey bee colonies. Exposure of foraging bees to contaminated pollen was relatively ubiquitous across the study apiaries. Overall, no clear relationships were observed between pesticide exposure and colony health and survival in our study, but we were not necessarily able to detect sub-lethal or interaction effects (Yang et al. 2008; Aliouane et al. 2009; Wu, Anelli and Sheppard 2011; Wu et al. 2012; Pettis et al. 2013).

Several of the most toxic insecticides detected among all sites were prescribed for use on corn and soybean, including chlorpyrifos, cyhalothrin, bifenthrin, and esfenvalerate. Casual observation of soybeans during bloom indicated that honey bees did not visit soybean flowers, although we did identify a small amount of soybean pollen from two sites (no corn pollen was detected) and, further, honey bees and wild bees have been documented visiting soybeans (e.g. Erickson 1975; Gill and O'Neal 2015). As further evidence of drift, we detected chlorpyrifos most prevalently (50%, 80%, and 63% of pollen samples, respectively) at sites D, E and F; the three sites with the most non-forage (primarily corn and soybeans) surrounding them.

Beekeeper-applied chemicals were some of the most prevalent chemicals detected in the pollen. This is somewhat surprising considering several of the chemicals (e.g. coumaphos, fluvalinate) have not been used by the beekeeper for over 5 years, and the beekeeper had a regular comb-replacement regime. Several of the compounds used in the past by beekeepers are lipophilic and tend to remain in wax comb for indefinite amounts of time (Wu, Anelli and Sheppard 2011). The detection of many in-hive miticides in forager pollen loads is likely due to these residues being present on the cuticles of most of the bees in the hives. This type of chronic exposure to pesticide residues can have myriad detrimental effects on bees (e.g. Haarmann et al. 2002; Pettis et al. 2004; Burley, Fell and Saacke 2008), and, further, has resulted in resistant populations of *Varroa* mites to many of the miticides in the beekeeper toolkit (Elzen et al. 1998; Pettis 2004).

4.3. Model utility and implications for future research

Our model indicates that if a beekeeper sought to achieve 80% survival based on uncultivated forage land alone, (s)he would require approximately 32,000-m^2 (32 hectares) of uncultivated forage land per hive (assuming pathogens and parasites are effectively controlled). This amounts to a total of approximately 15-km^2 of uncultivated forage land for an apiary consisting of 48 colonies. We observed survival of 75-88% occurring across a range of 9-47 hectares per hive. Further, if we consider that most uncultivated forage land is not completely covered in flowers (from our floral surveys of all sites and years, on average approximately 28% of uncultivated land contained flowers), the beekeeper would require a considerably smaller area of actual flowers over the entire growing season to achieve 80% survival based on land use alone.

Tools for long-term monitoring of honey bee colonies related to landscape factors have been developed in Western Europe (Odoux et al. 2014), and similar monitoring techniques considering colony level dynamics given land use trends over time and encompassing a large geographic region would provide valuable insight for beekeepers, researchers, and the future sustainability of bee-utilized landscapes in the US. Additionally, such land use quantification could be incorporated into existing efforts (e.g. national beekeeping survey, Bee Informed Partnership monitoring, National Pollinator Strategy) to better understand the role of land use, and changes in land use over time, in driving beekeeper apiary selection and colony health, productivity, and survival outcomes.

Further research is needed that hones in on targeted landscape and habitat enhancement effects, including cover types such as crop borders, restored prairies, alternative conservation program seed mixes, organic farms, cover crops, etc. Such research will contribute to greater resolution for beekeepers, thus affording them the ability to conduct “precision beekeeping” with respect to site selection and expected apiary performance based on land use. Here we have shown that selection of apiary sites based on land use by a beekeeper has value on predicting productivity and survival of colonies among apiaries. Therefore site selection is one critical factor that beekeepers, importantly, have control over to improve the productivity and survival of colonies in their operations.

5. Conclusions

We focused on the large-scale land use features of intensively-managed lands that are most utilized by honey bees to support colony productivity and, more importantly, colony survival to ultimately meet pollination contracts the following spring. We found that honey bee colonies positioned in agricultural lands utilize a high proportion of non-native, volunteer plants, as also shown by Requier et al. (2015) in France. However, unlike in the French system, there were relatively few areas of mass-flowering bee forage crops (i.e. rapeseed, sunflower) in our study area. Therefore, we suggest that bees in the NGP of the US are even more dependent on volunteer species of flowers present in uncultivated parts of the landscape than other more diverse cropping systems in the US or abroad. The nutritional demands of honey bee colonies during a pollinator crisis must be considered and weighed against the potential future ecological impacts of allowing certain non-native plants to grow in specific areas of the landscape. If such species are not allowed to be seeded or persist in critical regions for honey bees, then greater efforts are needed to identify and seed-in viable alternative, acceptable flowering plants on the landscape to support honey bee colonies.

Previous work has demonstrated the effects of land use on honey bee colonies under varying and alternative land use and beekeeping conditions. For example, Naug (2009) was one of the first to correlate course, large-scale land use to differences in colony losses by US state. Since that time, others have produced additional evidence suggesting that honey bees have a preference for, or most benefit from, agricultural lands compared to urban, forested, or mature grass lands (Clermont et al. 2015; Sponsler and Johnson 2015), or areas containing pollinator-conscious practices such as agri-environment schemes (programs incentivizing farmers) in the European Union (Couvillon et al. 2014).

Related, USDA conservation lands (voluntary landowner incentive programs) were prevalent near several of our apiary sites, and differences in observed floral coverage on such lands could have been due to several factors, including differences in program seed mixes, time the land was in the conservation program, weed and land management, and differences resulting from soil nutrients and water availability. Intriguingly, colonies from the three apiary sites with the highest amount of CRP lands nearby (A, C, and F) also collected the highest overall taxonomic diversity of pollen. However, care should be taken in assuming such federal programs are an automatic net gain for honey bee colony health and survival. Seed mixes should be utilized that are maximally beneficial to honey bees and other pollinators (and maintained to protect continued growth of forbs so as not to be outcompeted by grasses) if the goal is to significantly increase pollinator forage on the landscape.

Our focus here was on a large number of commercial honey bee colonies solely embedded in intensive agricultural lands for summer foraging, thus highlighting the delicate balance between high agro-ecosystem productivity and the availability of habitat for honey bee colonies required to meet national pollination service demands. In such landscapes, disparate sectors of the agricultural industry must coexist to provide healthy, reliable, and productive systems. Overall, this work provides an additional novel piece of evidence for the strong influence of land use within agricultural environments and the importance of the NGP for the performance and final outcomes of honey bee colonies that are part of the US commercial beekeeping industry. Recent land use and land use change in the NGP (Wright and Wimberly 2013), then, require closer attention to ensure habitat is available to a sustain large proportion of the commercial honey bee and pollination industry.

5. Acknowledgements

The authors would like to thank the collaborating beekeeper Zac Browning, USDA technician Nathan Rice, and USGS technicians Jordan Neau and Cali Roth. This project was funded by grants from USDA-NIFA and the North Dakota Department of Agriculture. The authors have no conflicts of interest to declare.

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TABLES

Table 1. Ground survey estimates of floral resources within land use categories.

			Sum total area flower type (m^2x10^3), 2010-12						
Land use	Site	Sum total area (m^2x10^3) land use 2010-12	Sweet Clover, *Melilotus* spp.	Alfalfa, *Medicago sativa*	Gum-weed, *Grindelia* spp.	Native Sunflower, *Helianthus* spp.	Sow-thistle, *Sonchus* spp.	Golden-rod, *Solidago* spp.	Proportion flower coverage
CRP	A	9627	1949	4172	117	487	672	664	0.837
	B	1950	-	975	-	-	-	-	0.500
	C	14093	463	892	162	1179	546	1282	0.321
	D	1058	143	413	36	71	-	36	0.660
	E	1264	322	64	64	44	-	193	0.545
	F	9210	683	56	136	287	574	126	0.202
Roadside Ditches	A	1477	261	109	108	85	43	233	0.567
	B	657	21	56	40	29	10	34	0.289
	C	1664	299	147	147	124	49	130	0.538
	D	1598	-	315	-	-	-	-	0.197
	E	2153	283	204	57	351	57	351	0.605
	F	1067	181	85	172	59	118	59	0.632
Fallow Land	A	1289	212	-	106	-	-	-	0.247
	B	1340	-	366	-	-	-	-	0.273
	C	-	-	-	-	-	-	-	-
	D	1706	-	132	-	-	-	-	0.078
	E	2524	22	-	11	-	11	-	0.017
	F	-	-	-	-	-	-	-	-
Grassland	A	2674	326	82	81	124	81	292	0.369
	B	33654	1652	720	637	417	332	1169	0.146
	C	883	-	31	31	31	31	31	0.177
	D	3637	-	-	-	-	-	-	0.000
	E	237	-	-	-	-	-	-	0.000
	F	323	-	-	-	-	-	16	0.075
Hayland	A	7062	1043	3539	102	53	54	-	0.678
	B	2994	-	2283	32	-	-	-	0.773
	C	3080	212	1805	97	203	97	97	0.816

			Sum total area flower type ($m^2 x10^3$), 2010-12						
Land use	Site	Sum total area ($m^2 x10^3$) land use 2010-12	Sweet Clover, *Melilotus* spp.	Alfalfa, *Medicago sativa*	Gum-weed, *Grindelia* spp.	Native Sunflower, *Helianthus* spp.	Sow-thistle, *Sonchus* spp.	Golden-rod, *Solidago* spp.	Proportion flower coverage
	D	2854	542	1248	-	-	57	-	0.647
	E	5918	51	308	179	78	-	-	0.104
	F	362	14	44	-	-	-	-	0.159
Pasture	A	43594	10664	562	4822	3708	1825	7204	0.660
Pasture	B	7631	86	21	106	21	3	137	0.049
Pasture	C	7761	0	115	639	262	-	703	0.221
Pasture	D	14874	-	5	97	-	-	-	0.007
Pasture	E	3451	231	252	527	-	-	371	0.401
Pasture	F	-	-	-	-	-	-	-	-

Table 2. Linear mixed effect models relating annual number of colonies surviving and honey yields across varying agricultural land use in North Dakota, 2010-2012.

Response	Model (log transformed m^2); Random effects: site and year	K	AICc	ΔAICc	*w*	Coefficients (95% C.I.)
Number of surviving colonies per apiary (3.2-km buffer)	Uncultivated forage	5	84.19	0.00	0.729	Intercept: -24.88 Uncultivated forage: 2.65 (1.20, 4.07)
	Uncultivated forage + Wetlands	6	87.85	3.66	0.117	Intercept: -19.14 Uncultivated forage: 2.75 (1.40, 4.16) Wetlands: -0.51 (-1.51, 0.49)
	Uncultivated + Cultivated forage	6	88.25	4.06	0.096	Intercept: -22.00 Uncultivated forage: 2.43 (0.82, 3.91) Cultivated forage: 0.06 (-0.10, 0.22)
	Cultivated forage	5	90.44	6.25	0.032	Intercept: 17.01 Cultivated forage: 0.07 (-0.11, 0.24)
	Wetlands	5	90.87	6.68	0.026	Intercept: 21.36 Wetlands: -0.26 (-2.18, 1.32)
	Uncultivated + Cultivated forage + Wetlands	7	93.09	8.9	0.009	Intercept: -17.83 Uncultivated forage: 2.57 (1.10, 4.09) Cultivated forage: 0.04 (-0.11, 0.20) Wetlands: -0.43 (-1.42, 0.53)
	Cultivated forage + Wetlands	6	95.03	10.84	0.003	Intercept: 19.50 Cultivated forage: 0.06 (-0.12, 0.23) Wetlands: -0.17 (-1.89, 1.42)
Honey production (3.2-km (buffer)	Uncultivated forage	5	152.32	0.00	0.389	Intercept: -99.66 Uncultivated forage: 8.44 (1.51, 16.15)
	Uncultivated forage + Wetlands	6	152.96	0.64	0.283	Intercept: -42.42 Uncultivated forage: 9.36 (3.17, 15.74) Wetlands: -4.96 (-9.69, -0.10)
	Wetlands	5	154.68	2.36	0.120	Intercept: 95.48 Wetlands: -4.11 (-9.61, 2.36)

Response	Model (log transformed m^2); Random effects: site and year	K	AICc	ΔAICc	*w*	Coefficients (95% C.I.)
	Cultivated forage	5	154.71	2.39	0.118	Intercept: 29.97 Cultivated forage: 0.64 (-0.30, 1.43)
	Uncultivated + Cultivated forage	6	156.44	4.12	0.050	Intercept: -82.47 Uncultivated forage: 7.19 (-0.67, 15.30) Cultivated forage: 0.32 (-0.63, 1.11)
	Cultivated forage + Wetlands	6	157.97	5.65	0.023	Intercept: 79.59 Cultivated forage: 0.53 (-0.31, 1.44) Wetlands: -3.35 (-8.51, 2.45)
	Uncultivated + Cultivated forage + Wetlands	7	158.49	6.12	0.018	Intercept: -40.10 Uncultivated forage: 9.05 (1.89, 15.90) Cultivated forage: 0.07 (-0.78, 0.95) Wetlands: -4.82 (-9.66, -0.19)

K represents the number of parameters; ΔAICc represents the difference between AICc values of each model and the top-ranking model; *w* is the AICc model weight.

7. Figure Captions

Fig. 1. Proportion of land use area within 3.2-km radius of each apiary, 2010-2012. Categories include (from bottom to top): 1) Uncultivated forage land use: CRP, pasture, fallow, grassland, hay land, roadside ditch (green), 2) Cultivated forage land use: Canola, sunflower, alfalfa (orange), 3) Wetlands (blue), and 4) Non-forage: Corn, soybeans, wheat, and oats (grey).

Fig. 2. Linear regression of area (m^2) uncultivated forage land on annual apiary survival (2a) and honey production (2b), and linear regression of annual honey production on survival (2c). ANOVA analysis of survival (2d) and honey production (2e) by site.

Fig.3. Pollen taxa and pesticide residues detected seasonally among the six study apiaries, 2010-2012. Pollen taxa are reported as the proportion (including unidentified pollen = undetermined) from each apiary on each sample date. Pesticide residues are reported as the $\log_{10}(x+1)$ hazard quotient values (ppb for each chemical/contact LD_{50}).

Fig. 4. Shannon-Weiner diversity index of land use (circles) surrounding apiaries and pollen taxa (triangles) identified in returning forager pollen loads, 2010-2012.

8. Appendix

Additional Supporting Information may be found in the online version of this article.

Table A.1. Land use areas quantified (m^2 $x10^3$) among the six study apiaries over three years, 2010-2012, in North Dakota. These raw categories were subsequently grouped into 1) Uncultivated forage land use: CRP, pasture, fallow, grassland, hay land, roadside ditch, 2) Cultivated forage land use: Canola, sunflower, alfalfa, 3) Wetlands, and 4) Non-forage: Corn, soybeans, wheat, and oats.

Table A.2. Pollen identification and proportion of taxa of detected pollen by site (A-F) and year (2010-2012).

Table A.3. Linear mixed effect modeling of survival and honey production relative to land use at alternative spatial scales.

Table A.4. Pesticides detected in forager pollen loads, 2010-12.

Fig. A.1. Map of apiary locations. Colonies were located in North Dakota from May-September and transported to California in October to overwinter and for almond pollination in February-March.

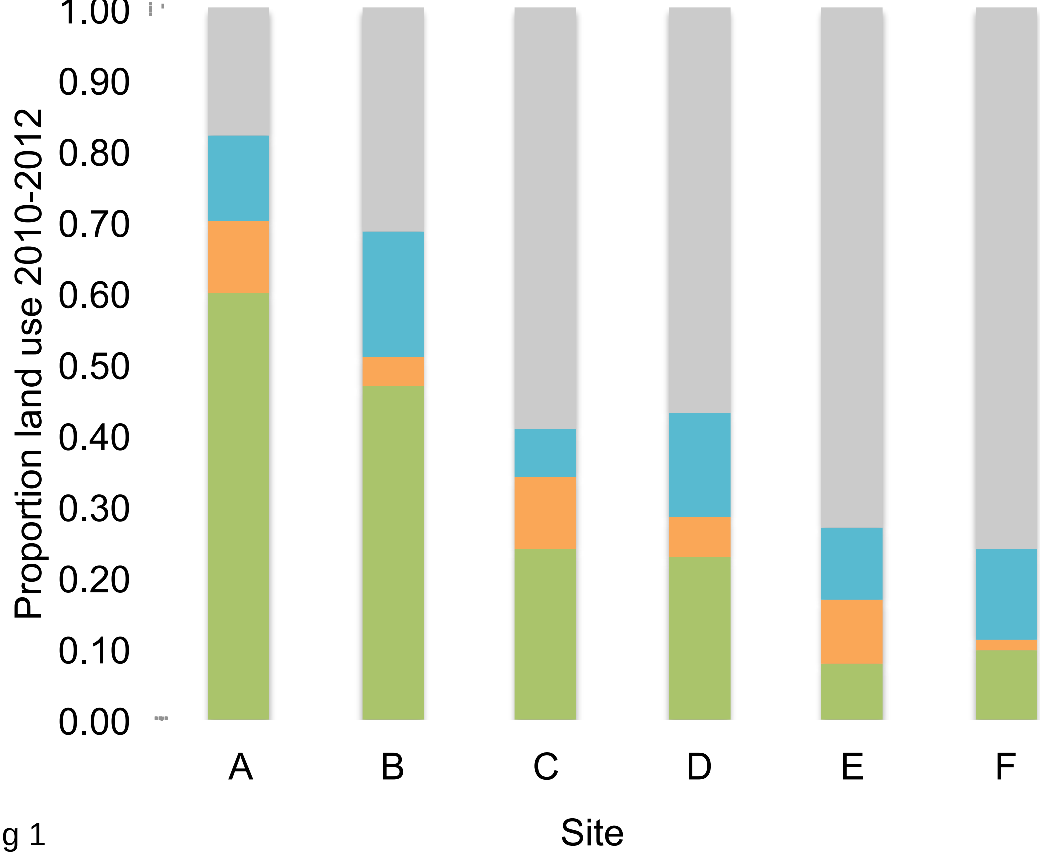

1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Proportion land use 2010-2012
A
B
C
D
E
F
Site

fig 1

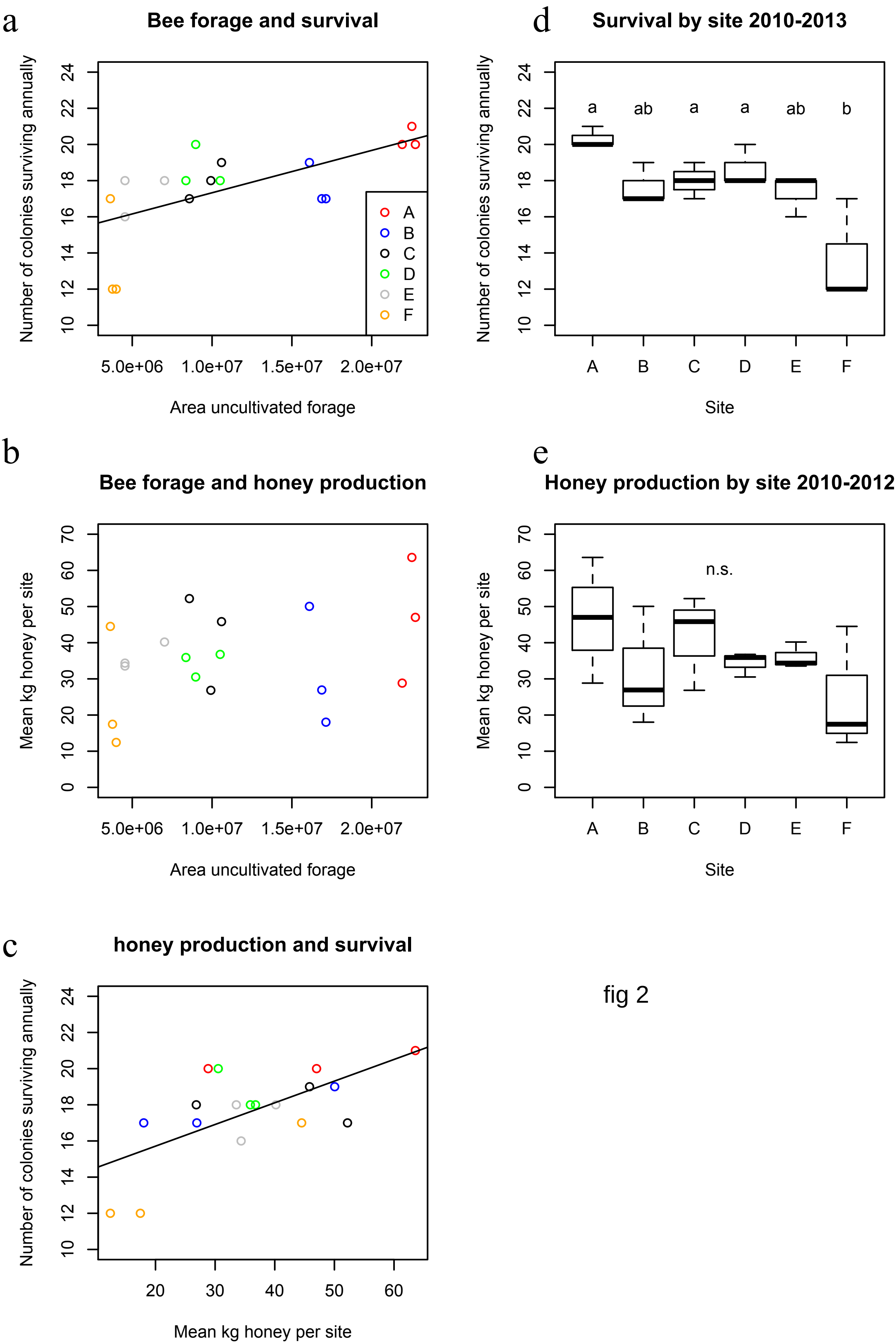

a
Bee forage and survival
Number of colonies surviving annually
10 12 14 16 18 20 22 24
5.0e+06 1.0e+07 1.5e+07 2.0e+07
Area uncultivated forage
A
B
C
D
E
F
b
Bee forage and honey production
Mean kg honey per site
0 10 20 30 40 50 60 70
5.0e+06 1.0e+07 1.5e+07 2.0e+07
Area uncultivated forage
c
honey production and survival
Number of colonies surviving annually
10 12 14 16 18 20 22 24
20 30 40 50 60
Mean kg honey per site
d
Survival by site 2010-2013
Number of colonies surviving annually
10 12 14 16 18 20 22 24
a ab a a ab b
A B C D E F
Site
e
Honey production by site 2010-2012
Mean kg honey per site
0 10 20 30 40 50 60 70
n.s.
A B C D E F
Site

fig 2

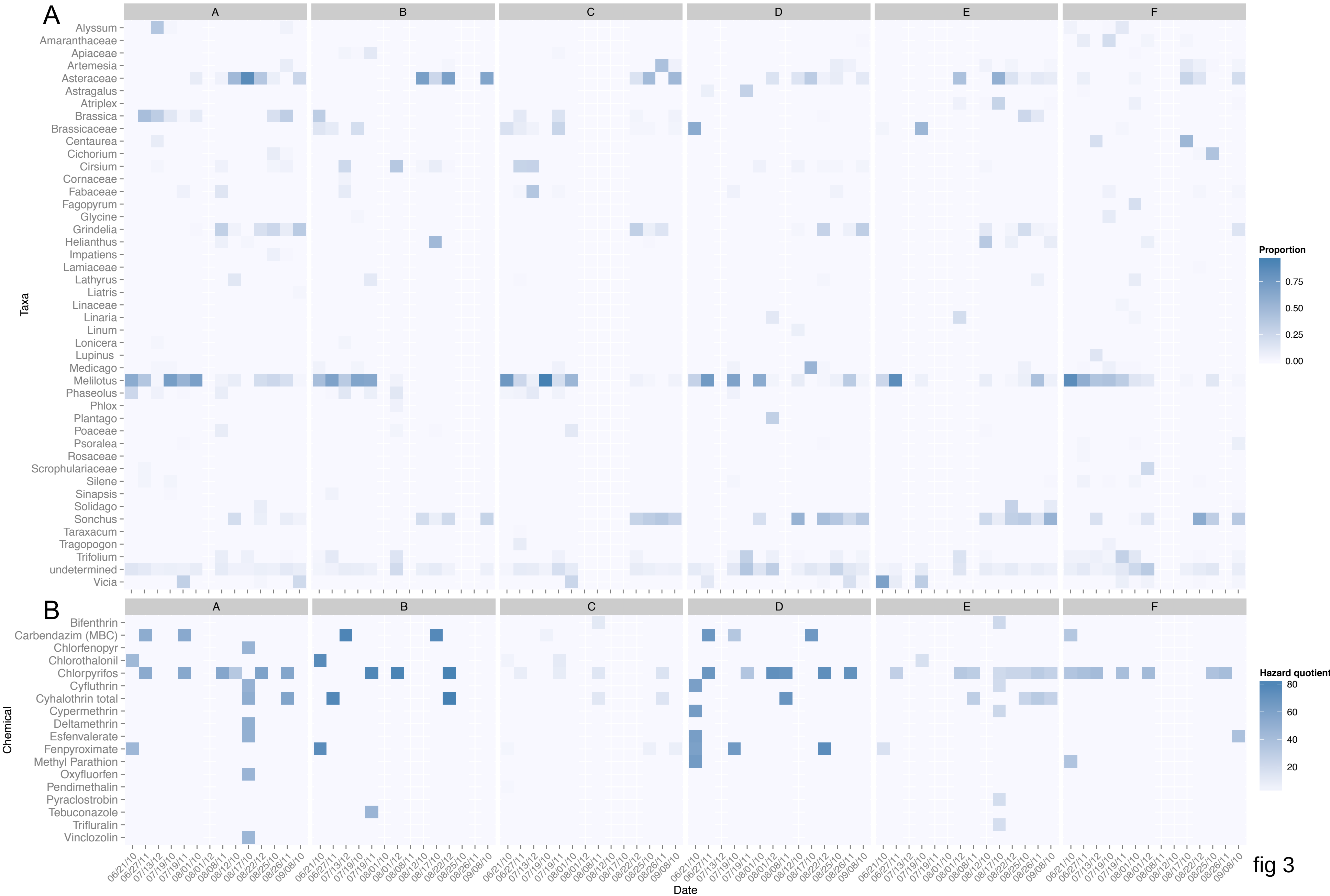
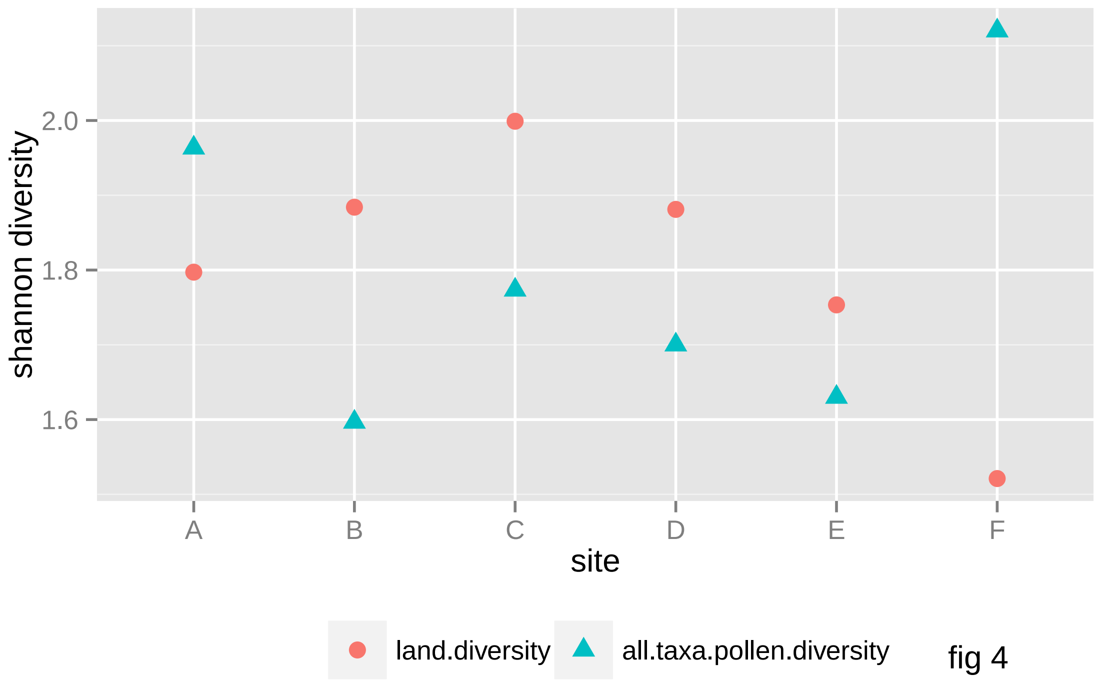

A
A
B
C
D
E
F
Alyssum
Amaranthaceae
Apiaceae
Artemesia
Asteraceae
Astragalus
Atriplex
Brassica
Brassicaceae
Centaurea
Cichorium
Cirsium
Cornaceae
Fabaceae
Fagopyrum
Glycine
Grindelia
Helianthus
Impatiens
Lamiaceae
Lathyrus
Liatris
Linaceae
Linaria
Linum
Lonicera
Lupinus
Medicago
Melilotus
Phaseolus
Phlox
Plantago
Poaceae
Psoralea
Rosaceae
Scrophulariaceae
Silene
Sinapsis
Solidago
Sonchus
Taraxacum
Tragopogon
Trifolium
undetermined
Vicia
Taxa
Proportion
0.75
0.50
0.25
0.00
B
Bifenthrin
Carbendazim (MBC)
Chlorfenopyr
Chlorothalonil
Chlorpyrifos
Cyfluthrin
Cyhalothrin total
Cypermethrin
Deltamethrin
Esfenvalerate
Fenpyroximate
Methyl Parathion
Oxyfluorfen
Pendimethalin
Pyraclostrobin
Tebuconazole
Trifluralin
Vinclozolin
Chemical
Hazard quotient
80
60
40
20
06/21/10
06/27/11
07/13/12
07/19/10
07/19/11
08/01/10
08/01/12
08/08/11
08/12/10
08/17/10
08/22/12
08/25/10
08/26/11
09/08/10
Date

fig 3



shannon diversity
2.0
1.8
1.6
A
B
C
D
E
F
site
land.diversity
all.taxa.pollen.diversity

fig 4