

Landscape distribution and abundance of animal-associated adult filth flies on commercial swine facilities in North Carolina, US

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Abstract. Filth flies associated with animal production transmit pathogens to humans and animals, propagate antimicrobial resistance in microbial communities and provoke nuisance litigation. Although dispersal of flies from facilities is often responsible for these negative effects, filth fly research on swine facilities has been limited to within the barns. Filth fly adaptations in space and time, as well as influences of abiotic and biotic factors impact distribution and abundance of animal-associated filth flies on swine production facilities. In this study, fly surveillance was conducted around four swine facilities in Bladen County, North Carolina, U.S.A. from January 2019 to October 2019. Traps were replaced weekly and animal-associated filth flies were identified. Flies were grouped for comparison based on biology and differences in pest management strategies. There were distinct differences in abundance and spatial distribution of different filth fly groups on the swine facilities, which are likely linked to environmental factors like spatial relation to crop production and species phenology. The impact of the observed temporal and spatial distribution and abundance is discussed in the context of filth fly management.

Key words. Calliphoridae, dispersal, fly control, insect, Muscidae, Sarcophagidae, swine.

Introduction

Animal facility-associated ‘filth flies’, or those developing in decaying organic matter and waste, have been linked to environmental movement of bacteria, fungi, parasites and viruses (Malik *et al.*, 2007). These flies have the potential to transmit enteric pathogenic bacteria (Onwugamba *et al.*, 2018) and antimicrobial-resistant bacteria (summarized in Onwugamba *et al.*, 2018), which is a concern for human and animal health.

Filth flies can develop in any animal facility, but confined animal feeding operations (CAFOs) are considered the main contributors to rural fly numbers (Thomas & Skoda, 1993; Winpisinger *et al.*, 2006). Swine facilities in the United States are typically considered CAFOs and can generate large amounts of accumulated waste and decaying organic matter that are suitable for pest fly development. Swine facilities with unmanaged

populations of filth flies dispersing into neighbouring residential areas may subject producers to litigation (Thomas & Skoda, 1993). Legal cases against animal production facilities citing general ‘flies’ as a cause of distress have increased over the past several decades, but without mentioning specific species or groups of flies. However, swine production often occurs in rural areas that support production of other animal commodities including beef cattle, poultry, and food and forage crops, which may impact fly populations and species distributions in the environment.

Species composition of filth flies can be affected by land use associated with livestock production (de Sousa *et al.*, 2020). Many filth fly species are sympatric and occupy a similar ecological niche. Different patterns of distribution in space (Brundage *et al.*, 2011) or time (Hwang & Turner, 2009; Weidner *et al.*, 2017) allow species to coexist in these areas.

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Changes in species distribution and numbers may be related to abiotic factors like climate or by biotic factors like habitat and management practices on a farm. However, the necessity of fly control or execution of control methods is limited by the understanding of filth fly seasonal dynamics and species composition.

House flies (*Musca domestica* (L.)) and black dump flies (*Hydrotaea (Ophyra) aenescens* Weidemann) (Diptera: Muscidae) are considered the major pest fly species associated with swine confinement housing (Burns & Nipper, 1960; Robertson & Sanders, 1979; Machtinger & Burgess, 2020). However, studies have been limited to evaluations of filth fly populations within the barns. There have been no evaluations of the animal-associated dipteran community distribution in the environment surrounding swine barns, including areas adjacent to barns, lagoons and effluent fields.

Control of fly populations in and around swine facilities is necessary to reduce negative impacts flies have on production (Rasmussen & Campbell, 1981; Catangui *et al.*, 1993, 1997), reduce the risk of pathogen transmission associated with flies in swine (Otake *et al.*, 2004; Schurrer *et al.*, 2004; Pitkin *et al.*, 2009) and reduce the risk of nuisance litigation. Health and legal concerns associated with filth flies require an assessment of species and species dynamics surrounding swine facilities to better understand filth fly diversity and abundance, as well as to inform control. The purpose of this study was to evaluate the distribution of filth flies surrounding commercial swine production barns in North Carolina, U.S.A.

Methods

This study was conducted from January to October 2019 at commercial swine facilities in Bladen County, North Carolina, in the southeastern coastal plain region of the state. Site 'Vacant' (34°29'16.80"N, 78°32'12.58"W) was a 5-barn sow facility that was cleaned and not used for animal production for the duration of this study. However, there was an active lagoon and effluent was sprayed on the surrounding corn fields. Site 'Sow' (34°29'56.44"N, 78°32'26.49"W) was a mixed-parity sow facility with seven barns including two farrowing barns, one breeding barn and one heat check barn, and two gestation barns as well as one finishing barn, which was used intermittently for facility culls. This facility had a maximum capacity of 4272 head and there were consistently animals in each barn throughout the year. The adjacent effluent fields were used to grow forage.

Sites 'Finishing 1' (34°29'49.11"N, 78°31'43.04"W) and 'Finishing 2' (34°30'17.90"N, 78°31'40.55"W) were swine finishing farms with barns using curtain ventilated side walls and a maximum capacity of 800–900 head per barn. Site Finishing 1 had four barns and Site Finishing 2 had three barns but were otherwise managed the same. Generally, animals were rotated out of the finishing house approximately every 25 weeks. For Finishing 1, houses were empty at the start of the study and swine were placed in February 21 and removed in June 25. Another cohort was added in July 23, which remained throughout the end of the study. For Finishing 2, swine were present in the houses at the start of the study and were removed

in May 9. A new cohort of swine was placed in May 14 and was removed in September 25. The houses of Finishing 2 remained empty throughout the completion of the study. Each house was cleaned between rotations. The adjacent fields at Finishing 1 and Finishing 2 were used for effluent spray, and beef cattle were rotated among these pastures throughout the year. Round hay bales were provided to the cattle in these fields in the winter and spring months. Round bale locations were rotated, but waste hay was not removed.

Manure was managed on all sites with shallow-pit systems that were flushed several times daily and emptied into an on-site waste storage treatment lagoon. Each site used conventional lagoon and spray management of effluent. After flushing from barns, effluent was treated via natural microbial processes. The stored wastewater was used to recharge the barn pits, and also periodically irrigated over on-site agricultural crops or pasture for nutrient enrichment purposes.

All sites were visited in December 2019 to evaluate for potential fly development locations. Fly development was not actively observed at any point during the study within the barns or in areas associated with the lagoons. Flushing was frequent and high volume; however, shallow pits were inaccessible, and development could have occurred in small areas of waste that were not reached by the flush. Carcasses were removed daily, housed in large freezers and disposed of weekly. During the study, fly control was not implemented outside of the best management practices for sanitation and animal welfare adopted by the producer.

BiteFree™ traps (Central Life Sciences, Schaumburg, IL) are constructed of folded wings of clear, polyethylene terephthalate (PET), adhesive treated plastic (16.3 cm in diameter by 34.7 cm in height). These traps were hung 1 m from the ground by securing the trap handle to a planter hook with gardening twine. BiteFree™ traps have been used to opportunistically capture house flies (Broce *et al.*, 1991; Geden, 2006; Zahn & Gerry, 2020) and other species of livestock-associated flies (Goulson *et al.*, 1999).

QuickStrike® Fly Abatement Strips (Central Life Sciences, Schaumburg, IL) were also used as odour targets for non-biting flies. The strips include an ampule of proprietary attractant and are composed of a plastic strip coated with sugar and the insecticide nithiazine. The ampule is crushed to release the attractant when the strips are deployed. These traps were hung on nails 0.7–1.0 m from the ground in a 2.5 × 2.5 cm pressure treated wood post and secured with garden twine. Nithiazine is inactivated rapidly by sunlight, so a 30-cm 'roof' of plywood was added to the top of the wooden post modelled after Geden (2005), which corresponds in surface area to the manufacturer recommendations of using a modified 19 L bucket as protection. An aluminium metal baking pan was placed beneath the strip and secured to the post with glue and duct tape to collect flies. The bottom of the pan was removed and replaced with window screen to allow for drainage.

Traps were placed 1 m apart at each monitoring station. Both traps were replaced on a weekly basis and flies in QuickStrike® trap pans were placed in vials with 80% EtOH for storage. Vegetation in a 1 m radius surrounding the traps was trimmed as necessary throughout the growing season. Traps and monitoring station images are provided in Fig. S1.

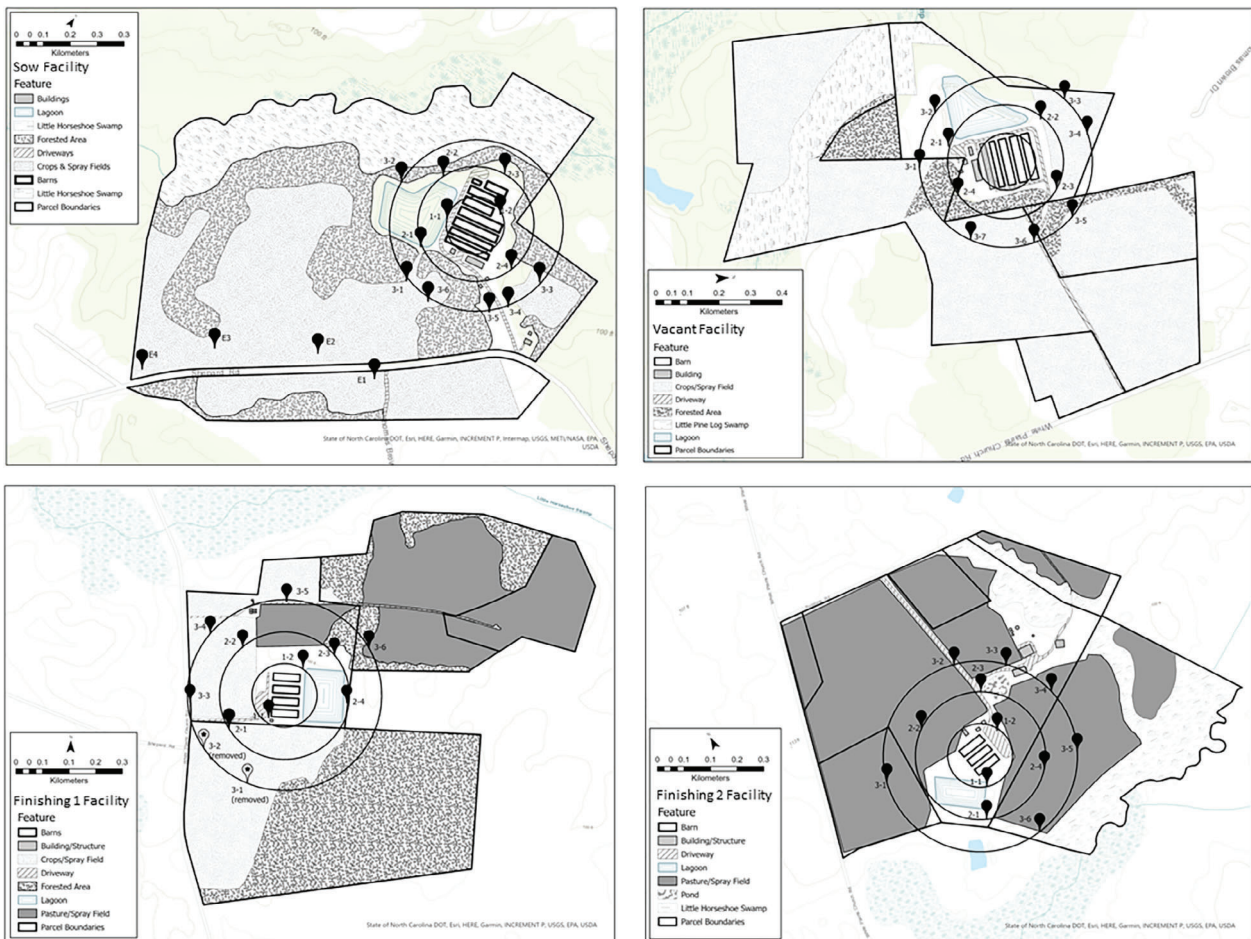


Fig. 1. Four swine facilities in Bladen County, North Carolina, U.S.A. were evaluated for animal-associated filth fly assemblages outside of swine barns. The Sow Facility was an 8-barn farrowing facility. Finishing 1 and Finishing 2 were finishing facilities with 4 and 3 barns, respectively. Vacant was a farrowing facility that did not have active production, but still had an active lagoon and effluent spray. Three circles of BiteFree™ and QuickStrike® traps were placed at each facility and an additional four traps outside the rings placed at the Sow facility. Two traps were initially placed at Finishing 1, but were removed due to farm management interference.

To evaluate fly distribution around each facility, traps were set in three concentric rings around each swine facility (Schoof *et al.*, 1952) (Fig. 1). The first ring was 75 m in diameter from the centre of each farm (the centre was determined to be the central point of each group of barns in each farm). The second ring was 150 m from the centre, and the third ring was 225 m from the centre. Two monitoring stations were located on the first ring, four on the second ring and up to seven on the third ring (Vacant); however, this was limited to six in Sow and Finishing 2 and to four on Finishing 1 due to property boundaries and facility activity (i.e., growing crops). In cases where monitoring stations were in pastures, a 5 m × 5 m electric fence was placed around the station to prevent interference from pastured animals. Sow had additional four traps ('E') that were placed in the crop fields in the direction of the neighbouring residential area. Uniform intervals between successive traps were not attempted as the primary criterion for monitoring trap location was suitable for fly capture. Monitoring stations were in sunny areas and consideration was given to sites that were least likely to be

manipulated by animals or people, not be inactivated due to excessive dust or effluent spray, and not be in the way of any facility maintenance or activities.

Fly identifications were limited to flies in the families Muscidae, Calliphoridae, Sarcophagidae and Tachinidae due to their frequent association with animals and animal production by-products. Captured flies were identified to genera using the keys in McAlpine *et al.* (1981). House flies (*M. domestica*) and black dump flies (*H. aeneascens*) were identified to species. Of the Calliphoridae, *Phormia regina* Meigen and *Cochliomyia macellaria* (Fabricius) were identified to species as they were the only species in their genus in this region of North America, but others were identified to genus only (*Lucilia* spp. and *Calliphora* spp.) because of volume of recovered samples. Sarcophagidae and Tachinidae are reported at the family level. Although taxa are not reported at the same biological level, we have grouped and compared the animal-associated fly taxa as they are generally considered for fly management and control based on their respective biology and pest status.

Statistical analysis

All analyses were conducted with R version 4.0.3 (R Core Team, 2018). Fly assemblages were evaluated by indices of dominance (Simpson index; Simpson, 1949), diversity (Shannon index; Shannon, 1948), evenness (Pielou's index; Pielou, 1966) and richness (Menhinick's index; Menhinick, 1964) to the level of genus, with all of the genera within the Sarcophagidae being represented by their family.

Shannon index (H') was calculated from the equation:

$$H' = - \sum (p_i \ln p_i),$$

where p_i is the proportion of total sample represented by genus i . Shannon index is a measure of community diversity and allows for mathematical comparisons among communities. However, it is generally affected by genera evenness and the presence of rare genera. It is often paired with the Simpson diversity index. A higher Shannon index value generally means a more diverse community.

The Simpson diversity index (D_s) was calculated from the equation:

$$D_s = 1 - \frac{\sum n(n-1)}{N(N-1)},$$

where n is the total number of organisms of a particular genus and N is the total number of organisms of all genera. The Simpson diversity index gives more weight to evenness among genera. The Simpson diversity index represents the probability of two individuals, taken at random from the same data set, being from the same genera. A higher Simpson diversity index value means greater diversity in that community.

Menhinick's index (D_{mn}) was calculated from the equation:

$$D_{mn} = \frac{S}{\sqrt{n}},$$

where S is the number of genera recorded and n is the number of genera in the sample. Menhinick's index is a measure of the number of genera within a sampling effort. A higher Menhinick's index value means the richness is generally higher, although sampling effort can affect this calculation. Nevertheless, it is a commonly used measure of richness in communities.

Pielou's index was calculated from the equation:

$$J' = \frac{H'}{H'_{max}},$$

where H' is derived from the Shannon diversity index and H'_{max} is the maximum possible value of H' . Pielou's index is a mathematical way to represent total counts of individuals in each genera. Pielou's index is thus a mathematical measure of evenness and can be used to compare different communities. A higher Pielou's index value means less variation in the abundances of each genera within a community.

The indices of Shannon, Simpson, Menhinick and Pielou, as well as abundance, were calculated from monthly totals of all trapping stations for each of the four farms using the 'diversity' function within the package 'vegan' (Oksanen

et al., 2012). A non-metric multidimensional scaling (NMDS) plot was generated for each farm using a Bray–Curtis dissimilarity matrix in two dimensions using the abundances from all traps within each trapping ring. In this case, the NMDS plots act to reduce multiple dimensions of species and trap location data into two dimensions that can be more easily interpretable, such as in principle component analysis (PCA). Thus, spatial proximity among species, traps and trap rings in each plot indicates how closely related these three factors are to each other. Ellipses were generated as 95% confidence intervals centred around the mean dissimilarity index (Bray–Curtis) between traps within each trap ring. A similar NMDS plot was generated representing all four farms at once, and the 95% confidence interval ellipses were centred around the mean dissimilarity of all traps within each farm. For the four farm NMDS, a non-parametric permutational multivariate analysis of variance (PERMANOVA) was conducted using the 'adonis' function also in the 'vegan' package. This tested the null hypothesis ($\alpha = 0.05$) that all central measures and dispersion around those measures among the four farms are equal within the two-dimensional space (Anderson, 2001). Thus, a P value < 0.05 indicates that not all farms resemble each other in terms of their relative abundance. For NMDS, stress level indicates how well the data are explained within the assigned multidimensional space and is considered of acceptable representation at < 0.2 , good representation at < 0.1 and excellent representation at < 0.05 (Clarke, 1993). Comparisons have been made using the respective identification groups: the two species that were identified within the family Muscidae (genera: *Musca*, *Hydrotaea*), the four genera identified in the family Calliphoridae (*Lucilia*, *Calliphora*, *Phormia*, *Cochliomyia*) and the specimens identified to the family Sarcophagidae.

Results

Abundance

Calliphoridae was the dominant family among three of the four farms, which all had animals present throughout the sampling duration (Sow, Finishing 1, Finishing 2) (Table 1). *Lucilia* was the dominant genus on the Sow facility, whereas *Phormia* dominated Finishing 1 and Finishing 2 farms. Sarcophagidae was dominant on Vacant, the farm that did not have any animals in production. *Musca domestica* recoveries ranged from 2.2% on Vacant to 18.4% on Finishing 2 of the total yearly captures and *Hydrotaea aenescens* ranged from 1.6% on Vacant to 13.5% on Finishing 1.

Mean total abundance of non-biting flies was highest in June on Finishing 1 (Fig. 2). Sow and Finishing 1 had the highest total abundances in April, respectively. Total abundance in Vacant appeared considerably lower than the other three farms from February through June. In January and then again from July to October, total fly abundance began to appear similar to the other facilities.

Trends appeared to differ in fly genera based on the sampling rings among the four farms. For Sow, *Lucilia* and *Musca* appear to be the most dominant genera in the first ring and peaked around April and May, respectively (Fig. 2). In the second and third rings, *Lucilia* and *Phormia* were the dominant genera

Table 1. Each family and genus of non-biting fly as a percentage of the total yearly captures on four commercial swine facilities (Sow, Vacant, Finishing 1 and Finishing 2) in Bladen County, North Carolina in 2019.

	Facility*			
	Sow	Vacant	Finishing 1	Finishing 2
Family Muscidae				
<i>Musca</i>	10.9%	2.2%	10.2%	18.4%
<i>Hydrotaea</i>	4.8%	1.6%	13.5%	7.6%
Total	15.7%	3.8%	23.7%	26.0%
Family Calliphoridae				
<i>Phormia</i>	24.1%	12.6%	27.7%	26.7%
<i>Lucilia</i>	31.7%	2.6%	8.4%	5.8%
<i>Calliphora</i>	1.0%	2.4%	6.0%	4.6%
<i>Cochliomyia</i>	1.8%	1.8%	4.3%	1.4%
Total	58.6%	19.4%	46.4%	38.5%
Family Sarcophagidae				
Various	25.9%	76.8%	29.9%	35.5%

*The Sow Facility was an 8-barn farrowing facility. Finishing 1 and Finishing 2 were finishing facilities with 4 and 3 barns, respectively. Greatest percentage for each farm is denoted in bold.

captured. Sarcophagidae abundance increased at the third and extended rings. In Vacant, *Phormia* was the most abundant genus, which peaked in March (Fig. 3). Sarcophagidae was also abundant at Vacant, and captures increased from the second to third rings. *Hydrotaea* was the single most abundant genus in the first ring of Finishing 1, peaking around May, but

Phormia was the most abundant in the second ring around April (Fig. 4). Both *Musca* and Sarcophagidae began to appear in greater numbers around June and September, respectively. In the third ring, *Phormia* peaked in April as in ring two. *Calliphora* peaked around May, and Sarcophagidae numbers increased in the third ring compared with the second ring around August. *Musca* was the most abundant genus in the first ring of Finishing 2 and among the most abundant in the second ring, peaking between May and June (Fig. 5). *Phormia* and Sarcophagidae appeared to be more abundant in the second and third rings compared with *Musca*.

Diversity and evenness indices

Shannon diversity and Simpson diversity peaked in different months based on farm (Fig. 6). For Sow, Shannon diversity peaked in May and June, with the lowest diversity in August. Simpson diversity also was the highest in May and June but lowest in August. Menhinick's index was the highest in February but the lowest in August, whereas Pielou's index of evenness was the highest in October but the lowest in August (Fig. 7). For farm Vacant, Shannon diversity and Simpson diversity were the highest in April but the lowest in August. Again, Sarcophagidae had very high relative abundance in August samples. Menhinick's index was the highest in April but the lowest in September and Pielou's index of evenness was also the highest in April but the lowest in September. Farm Finishing 1 had the highest Shannon diversity and Simpson diversity in June

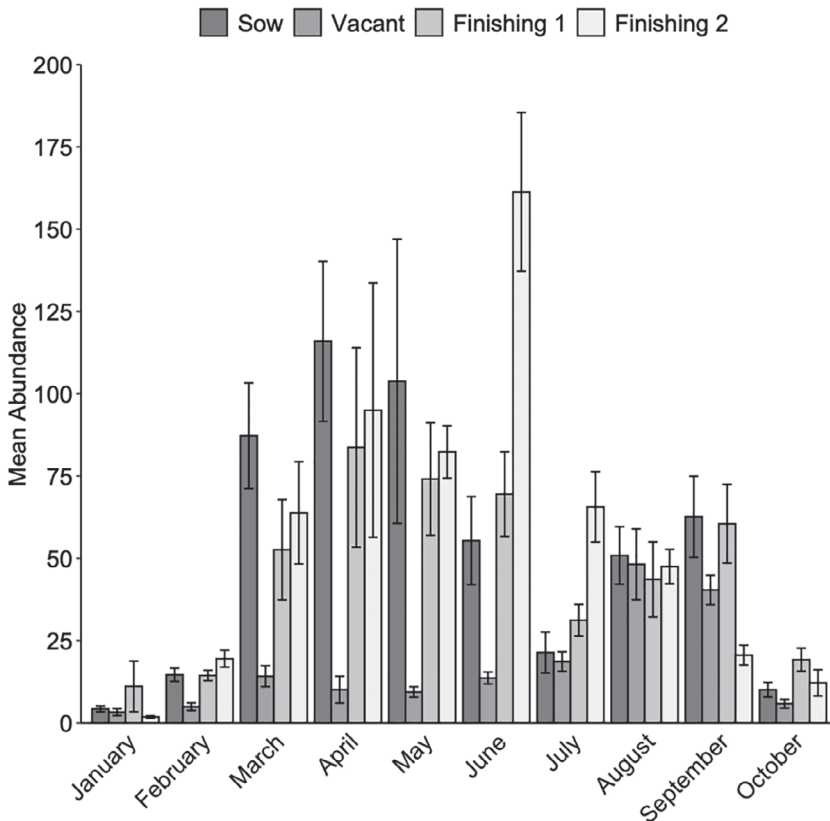


Fig. 2. Mean abundance of non-biting flies on the Sow, Vacant, Finishing 1 and Finishing 2 swine facilities in Bladen County, North Carolina in 2019. Error bars represent standard error of the mean.

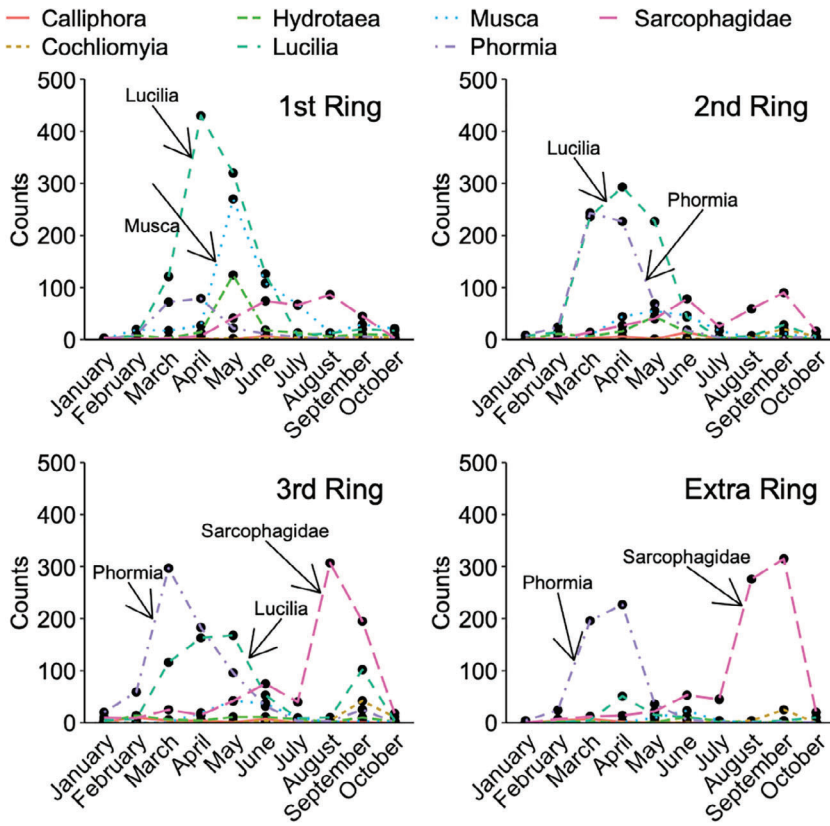


Fig. 3. Abundance of each non-biting fly genus or family captured within four sampling rings on the Sow facility in Bladen County, North Carolina in 2019. From a central point among the barns, first ring was 75 m away, the second ring was 150 m, the third ring was 225 m, and the extra ring traps were 520–920 m away.

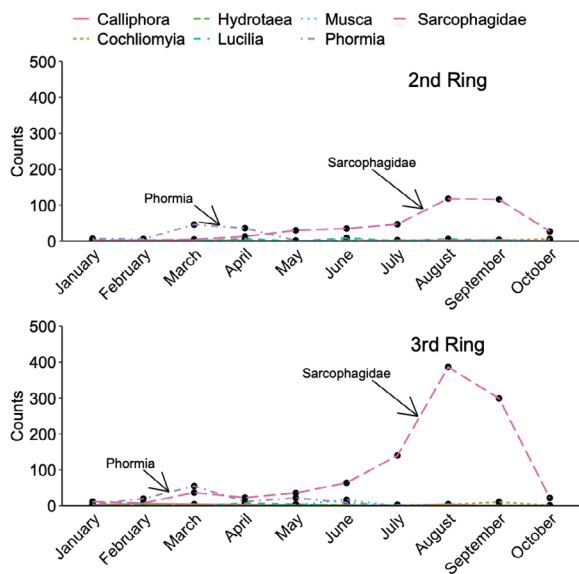


Fig. 4. Abundance of each non-biting fly genus and family captured within two sampling rings on the Vacant facility in Bladen County, North Carolina in 2019. The second ring was 150 m from a central point among the animal barns and the third ring was 225 m away.

and the lowest in January. Menhinick’s index was the highest in February but the lowest in March. Pielou’s index of evenness was the highest in January and the lowest in August. In farm

Finishing 2, both Shannon diversity and Simpson diversity were the highest in May. Shannon was the lowest in January, whereas Simpson was the lowest in March. Menhinick’s index was the highest in January and the lowest in June, whereas Pielou’s index was the highest in January and lowest in August.

Non-metric multidimensional scaling (NMDS)

There was a significant difference among the four farms with respect to relative abundance (Fig. 8; $R^2 = 0.400$, $F = 10.01$, $df = 3, 45$, $P = 0.001$). Matrices derived for each farm generally were well-represented by two-dimensional fit based on favourable stress values (Figs 9–13). Vegan: Community Ecology Package. R Package Version. 2.0-10 *Hydrotaea aenescens* was associated with the first trap ring on all active sites and *M. domestica* was associated with the first and second rings on all active sites. Other than *Lucilia* on Sow, there were no other fly associations with the first rings. *Calliphora* and Sarcophagidae were most closely associated with the third rings on all sites.

Discussion

Filth flies are often associated with CAFOs and some species; *M. domestica* in particular can become a residential nuisance as they disperse away from animal facilities (Thomas & Skoda, 1993). Increasing litigation and health concerns towards CAFOs resulting from the production of animal-associated filth flies

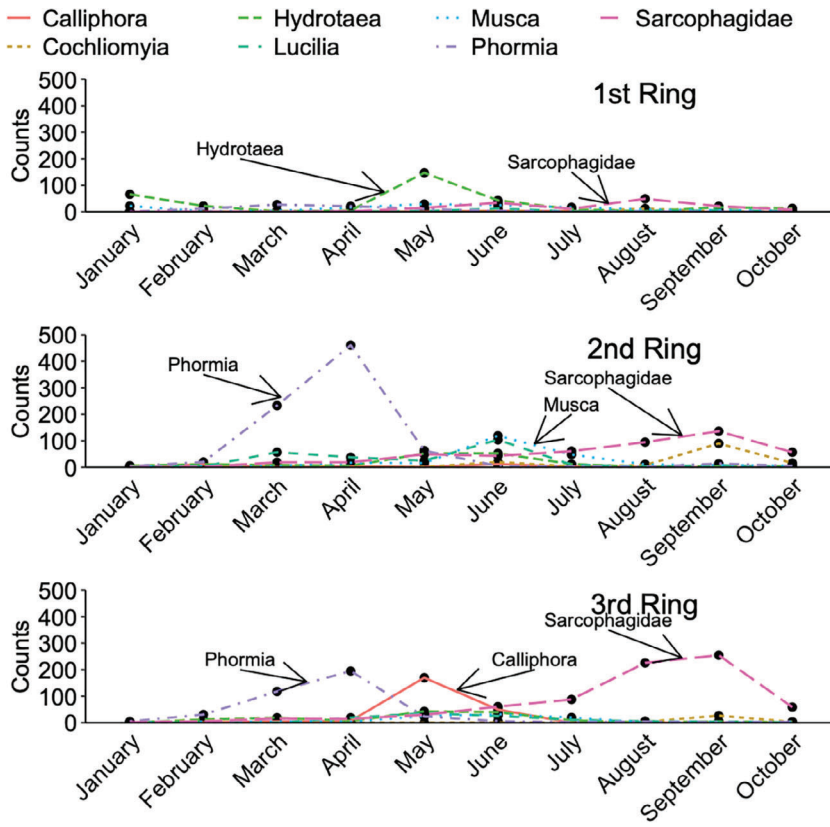


Fig. 5. Abundance of each non-biting fly genus or family captured within three sampling rings on the Finishing 1 facility in Bladen County, North Carolina in 2019. The first ring was 75 m away from a central point among the animal barns, the second ring was 150 m and the third ring was 225 m.

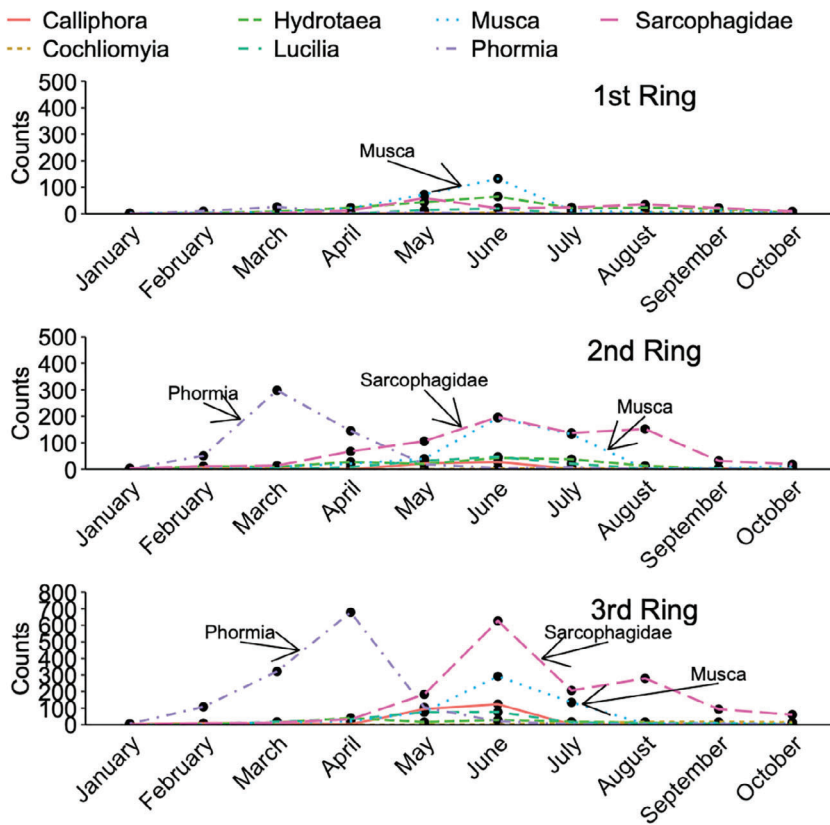


Fig. 6. Abundance of each non-biting fly genus or family captured within three sampling rings on the Finishing 2 facility in Bladen County, North Carolina in 2019. The first ring was 75 m away from a central point among the animal barns, the second ring was 150 m and the third ring was 225 m.

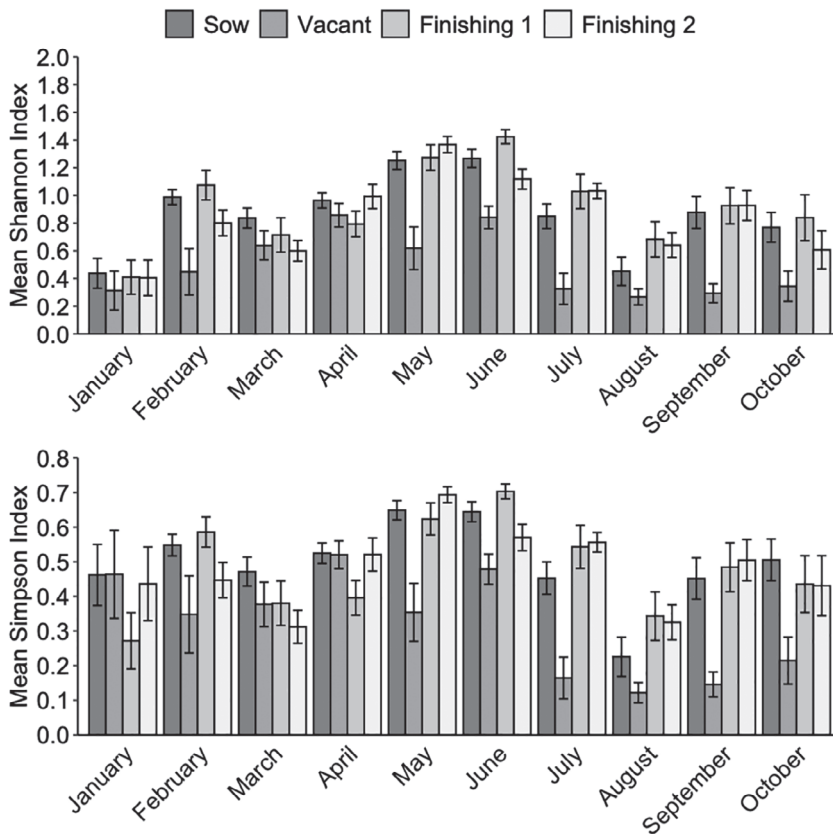


Fig. 7. Mean Shannon and Simpson indices for the non-biting fly genera and family on the Sow, Vacant, Finishing 1 and Finishing 2 swine facilities in Bladen County, North Carolina in 2019. Error bars represent standard error of the mean.

necessitates understanding the ecology of these flies on animal production facilities. In this study, the distribution, abundance and diversity of non-biting animal-associated filth flies were evaluated in the landscape of swine facilities in North Carolina. Overall, many genera of adult filth flies were identified, and the temporal and spatial distribution and abundance of these groups differed considerably. It is important to remember that this study was not designed to be a comprehensive survey of the dipteran biodiversity surrounding these facilities, but a comparison of the animal-associated filth flies to inform control practices.

There was distinct seasonality to the genera assemblages of Calliphoridae trapped over time. Some calliphorid genera, *Lucilia* and *Phormia*, were recovered earlier in the season, whereas *Calliphora* and *Cochliomyia* were found mid-summer into early fall. Similar early season abundance of *Phormia regina* has been documented in Florida and Michigan, U.S.A. (Gruner *et al.*, 2007; Babcock *et al.*, 2020), likely due to the intolerance of this species to warm temperatures (Byrd & Allen, 2001). Later season presence of *Cochliomyia macellaria* was documented by Weidner *et al.* (2017) in New Jersey, U.S.A. *Lucilia* and *Calliphora* seasonal abundance are more variable depending on location (Brundage *et al.*, 2011; Weidner *et al.*, 2017; Babcock *et al.*, 2020). Fluctuating temperatures associated with seasonality is known to influence Calliphoridae activity and phenology (Payne, 1965; Tabor *et al.*, 2004) and is likely the driver behind the different seasonal abundances observed in this study. The variability of *Lucilia* and *Calliphora* may be because multiple species could have been included in

these grouping that differentiate their niche temporally. A more focused study of these two genera may provide additional insight into species-specific phenology.

Interestingly, the greatest abundance of *M. domestica* and *H. aenescens* on all rings and sites was either in May or June. This is significant as it may indicate that production of these two species of flies, most associated with neighbourhood nuisance, was not necessarily related to on-farm production throughout the year. The Sow, Finishing 1 and Finishing 2 farms were operated on a year-round basis with minimal times that barns were without animals. It might be inferred that if the facilities were responsible for the production of these species, that fly populations would not rise and fall during the mid-point of the summer but instead would follow similar sustained patterns to other confined animal systems like poultry (Lysyk & Axtell, 1985) or experience extreme high and low population cycles related to specific management practices like barn cleanout (Stafford *et al.*, 1988). Months in which *M. domestica* and *H. aenescens* populations increased outside the barns happened to correspond with months that populations increased inside the barns found during a concurrent monitoring method study (Machtinger & Burgess, 2020). However, previous studies have documented fly movement between animal facilities in the same area (Lysyk & Axtell, 1986). It is impossible to determine if the recovered flies were present because of similar animal (swine, beef cattle, and poultry) production occurring throughout the local area encouraging fly immigration onto these swine facilities, or if fly production was occurring on site. Immature development of

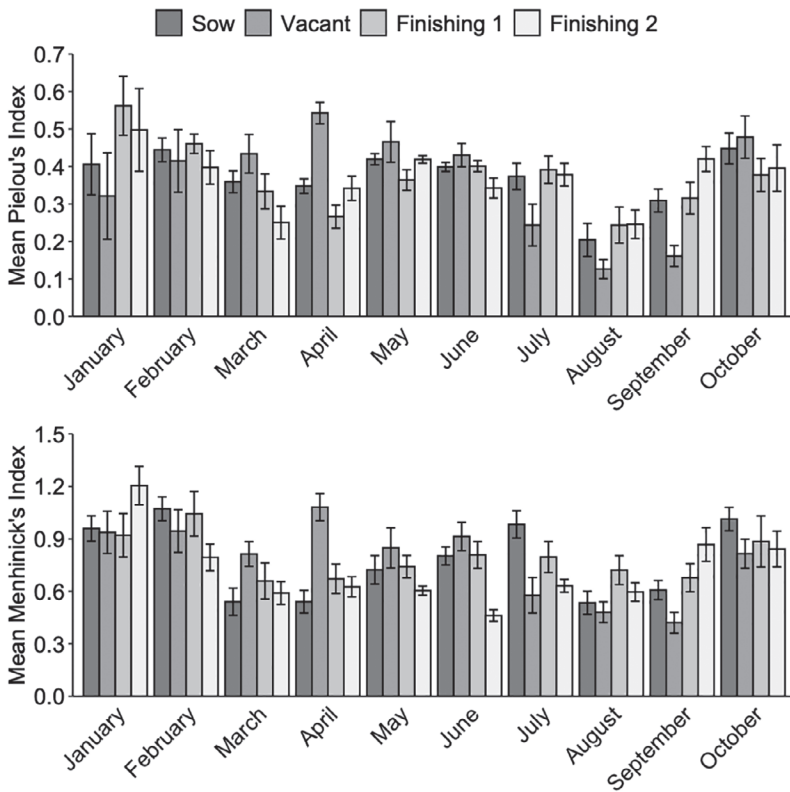


Fig. 8. Mean Pielou's and Menhinick's indices for the non-biting fly genera and family on the Sow, Vacant, Finishing 1 and Finishing 2 swine facilities in Bladen County, North Carolina in 2019. Error bars represent standard error of the mean.

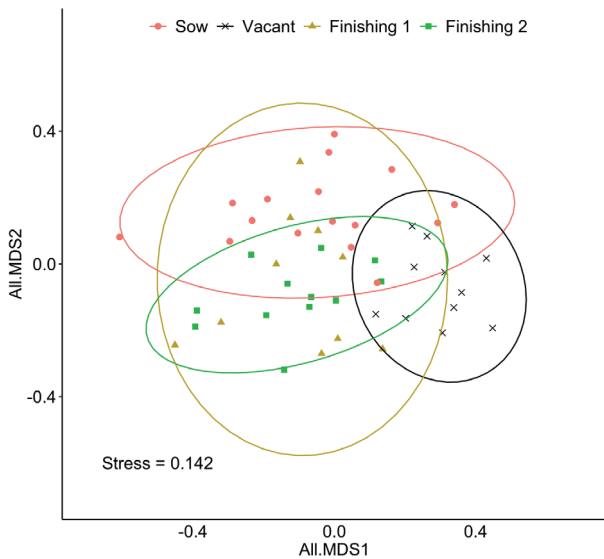


Fig. 9. Non-metric multidimensional scaling (NMDS) plot of Bray-Curtis dissimilarity among the Sow, Vacant, Finishing 1 and Finishing 2 facility trap sites in Bladen County, North Carolina in 2019. Ellipses represent 95% confidence intervals around each of the facilities.

M. domestica and *H. aenescens* was not observed within or outside of the barns during the study, but specific larval and pupal sampling efforts were not conducted for all species recovered.

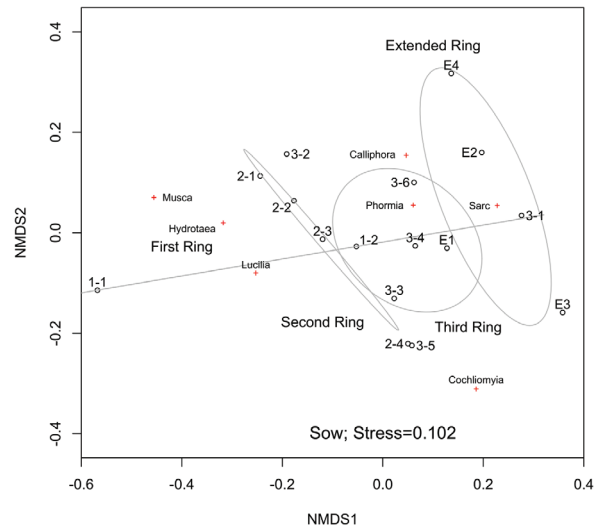


Fig. 10. Non-metric multidimensional scaling (NMDS) plot of Bray-Curtis dissimilarity among four rings on the Sow facility in Bladen County, North Carolina in 2019. Ellipses represent 95% confidence intervals around each ring. Trap sites are represented as the ring number to the left of the dash and the trap number within that ring to the right of the dash.

Within the evaluated fly groups, there was a pattern of increasing fly diversity across most sites throughout the year, with peak diversity in May and June. Generally, fly diversity was the lowest in August. This is likely due to early season flowering

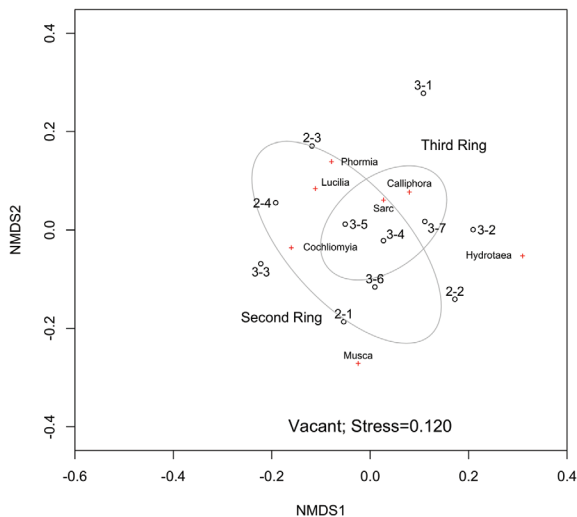


Fig. 11. Non-metric multidimensional scaling (NMDS) plot of Bray–Curtis dissimilarity between two rings on the Vacant facility in Bladen County, North Carolina in 2019. Ellipses represent 95% confidence intervals around each ring. Trap sites are represented as the ring number to the left of the dash and the trap number to the right of the dash.

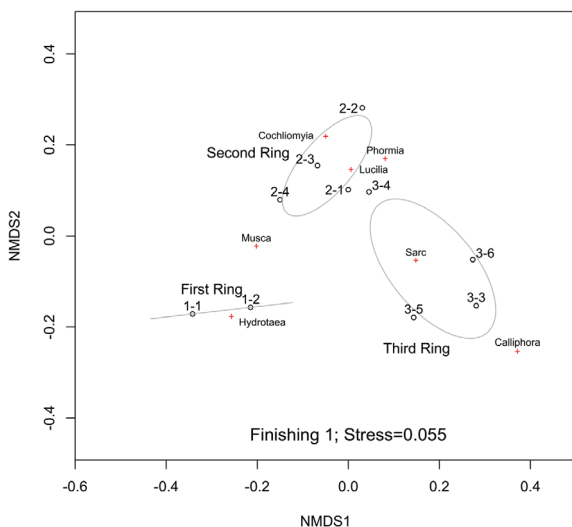


Fig. 12. Non-metric multidimensional scaling (NMDS) plot of Bray–Curtis dissimilarity between three rings on the Finishing 1 facility in Bladen County, North Carolina in 2019. Ellipses represent 95% confidence intervals around each ring. Trap sites are represented as the ring number to the left of the dash and the trap number to the right of the dash.

crops and the marked increase in Sarcophagidae specimens recovered in August with concurrent decreases in other fly species. The Vacant site was characterized by high numbers of Sarcophagidae and low *M. domestica* comparative to the remaining sites, which is illustrated by the lower diversity and dominance indices at this site. Although the Vacant site did not have animals present, it did have an active lagoon, effluent

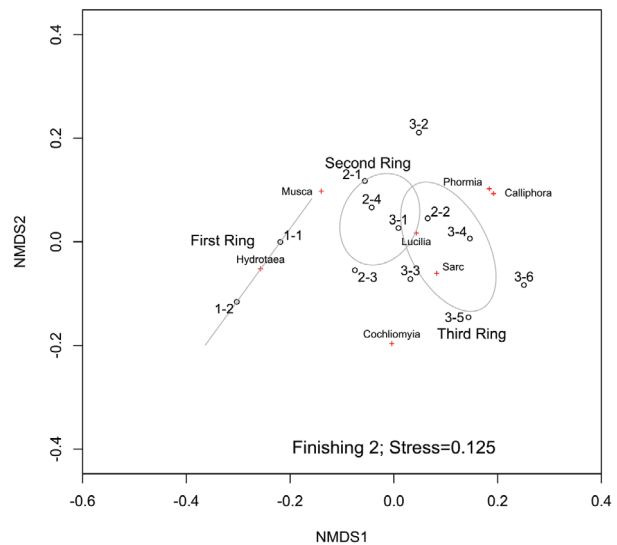


Fig. 13. Non-metric multidimensional scaling (NMDS) plot of Bray–Curtis dissimilarity between three rings on the Finishing 2 facility in Bladen County, North Carolina in 2019. Ellipses represent 95% confidence intervals around each ring. Trap sites are represented as the ring number to the left of the dash and the trap number to the right of the dash.

applied to crop fields and crops being grown as with the Sow site, so the lower indices may suggest that fly diversity could be directly associated with animal presence within the barns, and not necessarily landscape features, waste storage or other activities outside the barns. There is extensive evidence that saphrophagous Diptera use odours to locate oviposition sites (Liu *et al.*, 2016; Tang *et al.*, 2016). The concentration of odours from animals and animal-produced waste in the barns may be an attractant for many species of animal-associated flies, even if sanitation practices do not promote fly development.

Filth fly distribution on trapping rings was similar among the active sites (Sow 1, Finishing 1 and 2). *Hydrotaea aenescens* was associated with first ring trap captures. Similarly, *M. domestica* association was with the first and second rings, but there were very few recoveries from the third ring or the extra traps on Sow, suggesting that these two genera spatially relate to the animals more closely than other genera collected. *Hydrotaea aenescens* and *M. domestica* are associated with swine production (Burns & Nipper, 1960; Robertson & Sanders, 1979; Machtinger & Burgess, 2020). However, although *M. domestica* is known to disperse to neighbouring livestock facilities, *H. aenescens* infrequently disperses far from development sites (El-Dessouki & Stein, 1978; Nolan & Kissam, 1987). Interior barn monitoring of filth flies with spot and sticky cards at these farms during a concurrent study did not show fly levels above the threshold 100-spots or flies per card during most of the monitoring period on these study sites (Machtinger & Burgess, 2020). This, coupled with the restriction of *M. domestica* and *H. aenescens* to primarily the first and second trapping rings in the current study suggests that these two species did not disperse from the immediate vicinity of the swine barns during the sampling period.

The outer, third ring and extra traps were primarily associated with Sarcophagidae on all facilities and Sarcophagidae and *Calliphora* in the Finishing facilities. Although these flies can be associated with livestock because they develop in decomposing organic matter and carrion, they are not considered nuisance flies to the degree of *M. domestica* and *H. aenescens*. Diptera in the Calliphoridae and Sarcophagidae are important pollinators (Cook, 2020). Blow flies are considered the primary crop pollinating insect taxon outside of wild and managed bees (Heath, 1982; Cook, 2020). This may explain the association of these species with the second and third rings, which were often in fields of hay, corn, soy and other crops and had a wide variety of wildflowers and other flowering plants.

There were some limitations to the evaluation of these fly assemblages on swine facilities. The first is that the distribution of filth flies across the landscape is a result of fly spatiotemporal adaptations to coexist in similar ecological niches and is influenced by biotic and abiotic factors. The swine facilities evaluated in the current study were in a rural landscape, which included many neighbouring farms with crops, poultry, cattle and other swine facilities. In some cases, as with Finishing 1 and Finishing 2, beef cattle or other animals may be pastured on effluent fields. Species abundance and distribution may be related to management of these other facilities and animals. Assessment of species development in, and dispersal away from, a specific farm can be challenging to determine with these variables. It is important to recognize there may be many factors influencing filth fly assemblages on swine facilities and evaluating each situation for potential areas of fly development is necessary to inform control efforts.

The second limitation is that the trapping methods used were primarily for house flies (QuickStrike® traps) while the BiteFree™ traps allowed for opportunistic trap captures. Calliphoridae and Sarcophagidae surveillance is typically with carrion, so it is possible that abundance results might have been different with additional trapping methods. The volume of trap captures and degradation of specimens on the sticky BiteFree™ traps did not allow for species identification in some genera, which may have provided a more granular understanding of how these species interact in the rural landscape associated with swine production. In addition, abundance and diversity were evaluated in the context of animal-associated filth flies, which limited the range of species evaluated. Although only a few Muscidae species are typically a nuisance on swine facilities, a range of Calliphoridae species can be found depending on the situation. This should be taken into consideration and the results interpreted only in the context of animal-associated nuisance flies and not a greater assessment of dipteran diversity on the landscape.

In conclusion, there were several groups of animal-associated flies that were found on swine facilities that differed in dominance and abundance throughout the surveillance period. The primary fly groups associated with neighbourhood nuisance, *M. domestica* and *H. aenescens*, were found in association with the first and second rings and first rings, respectively, suggesting that these species remained close to production barns. In addition, changing abundance of this species by month suggested that the presence of *M. domestica* around the swine barns

may not be associated with farm production and increased biological or chemical control methods may not be warranted or effective at controlling these pests and the focus should be on cultural control methods. Although there was little evidence to suggest that filth flies of any one species were increasing in abundance to levels that may indicate nuisance or dispersing away from the swine production areas, it is important to note that many of these species can or may potentially be mechanical vectors for many pathogens associated with human and swine illness. Fly management efforts should concentrate on surveillance to identify fly pests and population fluctuations, and implementation of maximum sanitation practices both within and outside barns to reduce the risk of fly pathogen transmission.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. (A) Bite Free™ traps (background) and QuikStrike® Fly Abatement Strips (foreground) were used in monitoring stations to trap animal-associated filth flies at swine facilities in Bladen County, North Carolina in 2019. (B) When traps were in animal pastures, a 10 m × 10 m electric fence was installed to prevent animal interference.

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Author contributions

Author ETM designed the experiments, collected data, participated in data analysis and writing of the manuscript. Author JEB participated in experimental design, collected data and reviewed and approved the final manuscript. Author ERB, IV conducted the data analysis, participated in development of the manuscript and reviewed and approved the final manuscript.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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