

Airborne Environmental DNA Metabarcoding Detects More Diversity, More Efficiently, Than a Traditional Plant Community Survey

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Abstract

Background

Airborne environmental DNA (eDNA) research is an emerging field that focuses on the detection of species from their genetic remnants in the air. The study of airborne eDNA of plants has until now focused on single species detection, with no previous studies examining the entire plant community through metabarcoding. We therefore conducted airborne eDNA metabarcoding and compared the results to a traditional plant community survey.

Results

Over the course of a year, we conducted two traditional transect-based visual plant surveys alongside a yearlong airborne eDNA sampling campaign on a short-grass rangeland. We found that airborne eDNA detected more species than the traditional surveying method, although the types of species detected varied based on the method used. Airborne eDNA detected more grasses and forbs with less showy flowers, while the traditional method detected fewer grasses but also detected rarer forbs with large showy flowers. Additionally, we found the airborne eDNA method to be more efficient in terms of the time required to conduct a survey and able to detect more invasive species than traditional methods.

Conclusions

Overall, we have demonstrated for the first time that airborne eDNA can act as a sensitive and efficient plant community surveying method. Airborne eDNA surveillance has the potential to revolutionize the way plant communities are monitored in general, track changes in plant communities due to climate change and disturbances, and assist with the monitoring of invasive and endangered species.

Background

Accurate characterization of plant communities informs effective conservation, management, and restoration of communities and ecosystems (1). Scientists and managers have historically used field-based quadrats and transect methods to visually survey plant communities (2–3). With methods ranging from line-point intercept and belt transects to surveys as simple as visual identification, these traditional approaches can help to determine what plant species exist on a landscape (3). However, the quality of the results from traditional surveys rely heavily on how many resources (time and effort) are partitioned to the project (4). Additionally, traditional surveying relies heavily on an expert's ability to correctly identify plants. Furthermore, traditional plant community monitoring has the potential to damage or modify the plant life being surveyed and disrupt local animal populations, and such surveys can be labor-intensive and time consuming (3). Furthermore, by requiring someone to determine the identity of a species, these surveys can introduce inter-observer (different results from multiple observers) and intra-observer (same observer with errors over time) errors (5).

Genetic monitoring technology such as environmental DNA (eDNA) analysis can address these limitations, providing scientists and managers with a powerful tool for species detection. In this context, eDNA refers to the genetic material that is shed from an organism into its environment (6), and researchers can analyze bulk environmental samples such as water, soil, or air to determine whether that sample contains genetic material from a species of interest, which can provide clues about species proximity in space and time. Collecting bulk samples is often faster, cheaper, and requires less taxonomic expertise than searching throughout an environment for a potentially rare or hard to find species (7). Thus, eDNA methods are faster, less disruptive to the environment, and require less labor, which helps to directly address the limitations of traditional surveying methods (8–9). Additionally, multiple studies have found that eDNA methods have a higher sensitivity than traditional methods. For example, Jerde et al. (10) found that aquatic eDNA methods were more accurate than nets and electrofishing at locating Silver Carp (*Hypophthalmichthys molitrix*) and Bighead Carp (*H. nobilis*) in the Chicago Area Waterway System. Dejean et al. (11) also demonstrated that eDNA methods were more effective at detecting the invasive American bullfrog (*Lithobates catesbeianus*) in French wetlands compared to traditional auditory and visual surveys. Smart et al. (12) compared eDNA methods to traditional trapping techniques for the detection of an invasive smooth newt (*Lissotriton vulgaris vulgaris*) and found that eDNA detection probabilities were significantly higher than traditional trapping. Together, these and other studies emphasize the potential benefits eDNA analysis can provide to researchers and managers alike.

Historically, eDNA detection has been primarily applied to water (6, 13–14) and soil samples (15–17). The majority of airborne sampling has focused primarily on the detection of wind-borne grass pollen and its relation to human health (18–20). However, recent work from Johnson et al. (21–22) demonstrated that airborne eDNA can detect both anemophilous (i.e., wind pollinated) and non-anemophilous (e.g., insect pollinated) plant species. Additionally, Johnson et al. (21) found that not only could species be detected that were not wind pollinated, but also during a season when target species are not flowering and pollination is not occurring. Furthermore, Johnson et al. (23) found that airborne eDNA trends correspond to seasonal patterns and acute disturbances on a short-grass rangeland landscape. More recently, Aalismail et al. (24) found that airborne eukaryotic communities (including plant species) could be detected with samples from the global dust belt over the Red Sea. This indicates that airborne eDNA could be used in a similar manner to that of aquatic and sediment systems including community surveys, endangered species detection, and invasive species prevention. Therefore, we hypothesize that eDNA metabarcoding could be applied to survey entire plant communities.

Metabarcoding describes the process of using next-generation sequencing technologies to analyze eDNA samples and assess the total biodiversity of that sample, rather than the single-species, PCR-based approaches described so far (8). Next-generation sequencing technology is a massively parallel approach that allows researchers to quickly sequence thousands of sequences at once (25). As metabarcoding technology continues to improve and costs decrease, the field of eDNA research is shifting from single-species approaches toward using metabarcoding for a more comprehensive and efficient study of whole communities (7). Studies in aquatic systems have shown that metabarcoding can equal or exceed the performance of traditional field based methods. For example, in aquatic systems, Valentini et al. (26)

found that eDNA metabarcoding could more accurately detect both bony fish and amphibians compared to auditory, visual, and collection-based methods. Recently, McClenaghan et al. (27) used eDNA metabarcoding to detect deep sea fish and found that the eDNA method gave similar results to conventional field surveys while having a much lower sampling effort requirement. Additionally, eDNA metabarcoding has also been used for sediment analysis such as the study from Yoccoz et al. (16) where after metabarcoding, terrestrial sediment samples were shown to correctly detect present species at the surface. Additionally, Parducci et al. (28) used metabarcoding on lake sediment cores to detect the ancient plant communities that once lived in the area.

Metabarcoding of air samples has focused primarily on pollen and human health. For example, Korpelainen and Pietilainen (29) used metabarcoding to study the biodiversity of indoor pollen, how it changed over time, and its potential impact on human health. In a comparison of methods, Kraaijeveld et al. (20) found metabarcoding to be more effective at identifying mixed pollen grains than traditional microscopy. Leontidou et al. (30) developed protocols for processing and identifying pollen samples with metabarcoding. These previous works provide a foundation for airborne eDNA monitoring; however, with the understanding that airborne eDNA contains DNA from more than just pollen (21), an eDNA metabarcoding approach offers an opportunity to simultaneously study an entire plant community rather than limiting analysis solely on pollen. If airborne eDNA can be used as an effective plant community monitoring method, it could revolutionize the way plant communities are surveyed, invasive species are detected, and endangered species are monitored. Airborne eDNA metabarcoding may be able to detect species more efficiently, with less disturbance, and less effort than traditional surveying. A metabarcoding analysis would also allow us to examine the ecology (origin, state, transport, and fate) of airborne eDNA and how signals change over time to help produce better studies in the future (31). However, no research has examined metabarcoding as a terrestrial plant community monitoring method or compared this to traditional plant surveying methodology.

Therefore, we provide the first comparison of airborne eDNA metabarcoding and traditional plant survey methods in a field setting. To understand how airborne eDNA metabarcoding could be useful for plant community surveys, we compared airborne eDNA and traditional plant community surveys over the course of a year to capture the growing and flowering seasons of the species on our study site. The goal of this research was to understand whether and how airborne eDNA metabarcoding might be used as a plant community survey tool and how the results compared to a traditional method. Specifically, we wanted to 1) Understand which method (metabarcoding or traditional) detected the most species, was more efficient, and what types of species were found; and 2) Examine spatial and temporal patterns in airborne eDNA signals over the course of our yearlong survey.

Results

Traditional survey

Over the course of a year, we conducted two traditional transect-based visual plant surveys (September 2018 and May 2019) at the Texas Tech University Native Rangeland consisting primarily of short-grass rangeland habitat (Fig. 1). Survey methods included a transect-based, line-point intercept survey and broader visual survey methods. The traditional plant community survey conducted in September found a total of 56 species, and the May survey identified 93 species. Overall, when both the September and May surveys were combined, a total of 103 unique species were detected (Appendix 1). There were 23 specimens from the two surveys unidentifiable due to the plant species being too young or damaged beyond recognition. Furthermore, for the comparison between both survey methods, 23 species that were found with the traditional plant survey did not have sequences available in Genbank and thus were removed from the comparison since they constitute an artifact of the analysis. In other words, we had no way of knowing if those species were detected with eDNA or not since there was no reference to compare our raw data to. This means that in total, the traditional survey found 80 species that could be compared to the eDNA metabarcoding survey. Of the 80 species that were found with the traditional methods, we found 63 forbs (79% of total), 15 grasses (19% of total), and 2 trees (2% of total; Fig. 2a). Across both traditional survey events, the three most common species were *Bouteloua gracilis*, *Prosopis glandulosa*, and *Laennecia coulteri*. For the September survey, the three most common species were *Bouteloua gracilis*, *Laennecia coulteri*, and *Prosopis glandulosa*, and the three most common species for the May survey were *Bouteloua gracilis*, *Prosopis glandulosa*, and *Salsola tragus* (Table 1). Of the most commonly detected species in traditional surveys, *Erigeron modestus*, *Solanum elaeagnifolium*, *Quincula lobate*, and *Ammoselinum popei* were not able to be included in the reference library for the metabarcoding survey as there were no sequences available.

Table 1

The most common species found for both surveys and the September and May surveys individually during the traditional survey method. The three species in bold were not included in the reference library for the metabarcoding method as there were no sequences in Genbank.

Traditional Survey Most Common Species		
Total	September	May
<i>Bouteloua gracilis</i>	<i>Bouteloua gracilis</i>	<i>Bouteloua gracilis</i>
<i>Prosopis glandulosa</i>	<i>Prosopis glandulosa</i>	<i>Laennecia coulteri</i>
<i>Laennecia coulteri</i>	<i>Salsola tragus</i>	<i>Prosopis glandulosa</i>
<i>Helianthus ciliaris</i>	<i>Helianthus ciliaris</i>	<i>Helenium amarum</i>
<i>Helenium amarum</i>	<i>Solanum elaeagnifolium</i>	<i>Erigeron modestus</i>
<i>Erigeron modestus</i>	<i>Euphorbia lata</i>	<i>Lepidium densiflorum</i>
<i>Lepidium densiflorum</i>	<i>Kochia scoparia</i>	<i>Helianthus ciliaris</i>
<i>Salsola tragus</i>	<i>Hopia obtusa</i>	<i>Teucrium laciniatum</i>
<i>Solanum elaeagnifolium</i>	<i>Quincula lobata</i>	<i>Ammoselinum popei</i>
<i>Teucrium laciniatum</i>	<i>Portulaca oleracea</i>	<i>Plectocephalus americanus</i>

Environmental DNA Metabarcoding Survey

We deployed Big Spring Number Eight (BSNE) dust traps at the center of each traditional plant survey location (N = 9; Fig. 1) to collect airborne eDNA for comparison with traditional survey results. Since airborne eDNA collection is less labor intensive than traditional surveys, we recovered eDNA from traps approximately every two weeks for the same year represented in our traditional plant surveys (i.e., traps were deployed from June 11th 2018 until June 14th 2019, with 22 collection events in between). We sequenced the plant nuclear ITS2 gene from collected DNA using an Illumina MiSeq platform to compare airborne eDNA-based method performance with traditional surveys. Across all samples and PCR plates, no contamination was detected in non-template controls and extraction blanks. To ensure that the maximum number of species were detected for our eDNA survey, we performed both a reference library analysis and a BLAST analysis to confirm any species not on the original study site list were not missed. The reference library method was able to detect 81 species while the BLAST analysis found an additional 10 species. Overall, between both the reference library and BLAST survey, a total of 91 species were detected (Appendix 1). Of these 91 species, we found 61 forbs (67% of total), 26 grasses (29% of total), and 4 trees (4% of total; Fig. 2b).

A reference library-based approach relates sequences to an existing, curated database. Thus, by curating a database of all species known to occur historically on our study site, we could compare sensitivity of eDNA-based and traditional survey approaches for detection of a known universe of plant species. Thus,

eDNA analyzed from Event 9 detected a total of 39 species from our curated reference library, while Event 21 samples included 46 species. Across all samples, we recorded a total of 127,761 reads. From those 127,761 reads, the top three species found within our reference library for all our samples were *Prosopis glandulosa*, *Salsola tragus*, and *Descurainia pinnata* (Table 2). The Event 9 samples produced a total of 14,517 reads and found that the three most common species were *Bouteloua gracilis*, *Salsola tragus*, and *Kochia scoparia* (Table 2). The Event 21 samples on the other hand had 63,114 reads and found that *Prosopis glandulosa*, *Salsola tragus*, and *Descurainia pinnata* were the top three species detected (Table 2). For our study site, the wind predominantly blows from west and northwest down to the east and southeast. We found that both Event 9 and Event 21 shared similar patterns with the lowest amounts of species being detected in the northwesterly traps while the largest number of species were found within the center of the rangeland (Fig. 3). Additionally, we calculated how many species were detected by the combined eDNA samples (22 sampling events) across the entire year to determine patterns and trends in the number of species detected (Fig. 4).

Table 2

The most common species found for the eDNA metabarcoding reference library analysis. This includes the total species, the samples that corresponded to the September survey and the May survey as well.

Reference Library Metabarcoding Most Common Species		
Total	Event 9	Event 21
<i>Prosopis glandulosa</i>	<i>Bouteloua gracilis</i>	<i>Prosopis glandulosa</i>
<i>Salsola tragus</i>	<i>Salsola tragus</i>	<i>Salsola tragus</i>
<i>Descurainia pinnata</i>	<i>Kochia scoparia</i>	<i>Descurainia pinnata</i>
<i>Bouteloua gracilis</i>	<i>Cynodon dactylon</i>	<i>Helenium amarum</i>
<i>Helenium amarum</i>	<i>Gutierrezia sarothrae</i>	<i>Laennecia coulteri</i>
<i>Ulmus pumila</i>	<i>Ambrosia psilostachya</i>	<i>Machaeranthera tanacetifolia</i>
<i>Kochia scoparia</i>	<i>Sporobolus cryptandrus</i>	<i>Aphanostephus ramosissimus</i>
<i>Laennecia coulteri</i>	<i>Sorghum halepense</i>	<i>Oxalis dillenii</i>
<i>Cynodon dactylon</i>	<i>Laennecia coulteri</i>	<i>Cynodon dactylon</i>
<i>Machaeranthera tanacetifolia</i>	<i>Verbesina encelioides</i>	<i>Ratibida columnifera</i>

To examine if any species not on our reference library were detected with our eDNA analysis, a BLAST survey was also completed. The BLAST survey results required more interpretation than the reference library results. The BLAST survey results used a total of 144,155 reads to identify 649 ASVs. Despite having 649 ASVs, only 178 were able to match 100% to a species or genus. From these 178 successfully matched ASVs, a total of 73 species or genera were identified. For our comparison the ASVs that could only identify samples to genus level were removed from the comparison total values, since no single

species could be determined. Of the remaining species, ten were unique species that were added to the 81 species from the reference library. While our study site is isolated within the city of Lubbock, there are several agriculture fields near our site, a golf course, and several residential living areas that contributed airborne eDNA. For example, the BLAST survey identified several agricultural crops that exist within a mile of our study site such as cotton (*Gossypium hirsutum*) and soybean (*Glycine max*). Additionally, the BLAST survey identified several genera and species of trees that are planted throughout the golf course and residential areas surrounding our study site. This includes the *Pinus* (Pine trees) genus, *Platanus* (Sycamore) genus, cottonwood (*Populus deltoids*), and several oak species (*Quercus* sp.). These species represent real signals and real species in the environment, but for the sake of surveying our study site they were not included in our comparison.

Comparison

Overall, our traditional survey found a total of 80 species while the eDNA metabarcoding surveying found 91 species. The types of species (grasses, forbs, trees) that each survey detected were found to vary based on the method being used (Fig. 5). The eDNA metabarcoding survey and traditional survey shared the identification of 13 grasses while the eDNA survey solely detected an additional 13 species compared to just 2 grass species solely detected with the traditional survey (Fig. 5a). Both surveys combined to find 40 shared forbs while our eDNA survey detected 21 unique forbs compared to the 23 detected solely by the traditional survey (Fig. 5b). Lastly, both methods found 2 trees while the eDNA survey detected an additional 2 unique species (Fig. 5c).

Discussion

Our goal was to determine how an airborne eDNA metabarcoding survey performed compared to a traditional plant community survey. We've demonstrated that the airborne eDNA survey was able to detect more species than the traditional survey. We found that both surveys performed differently, finding different amounts and types of species. Additionally, we gained knowledge about the ecology of airborne eDNA and how natural factors, such as weather, impacted our ability to collect airborne eDNA.

Traditional survey compared to eDNA survey

We found that the airborne eDNA survey (reference library and BLAST) was able to detect more species than the traditional surveying methods, detecting a total of 91 species compared to the 80 species found by the traditional survey. While the airborne eDNA metabarcoding survey found more species, the two surveys varied in the species found, with each method identifying species that were missed by the other and differing greatly in the types of plants detected (Fig. 5). The airborne eDNA survey found 13 grasses not seen in the traditional surveying methods compared to only 2 grasses found with traditional methods not seen in the eDNA survey (Fig. 5a). Grasses predominantly utilize wind pollination, which would release a lot of genetic material and explain the relatively high performance of the eDNA method compared to a traditional survey. Additionally, grasses can be particularly challenging to detect with

traditional surveying methods (20), which further explains why eDNA methods would outperform traditional survey methods for this group of plants.

More surprisingly, the eDNA survey detected 21 forbs not seen in the traditional survey compared to 23 forbs detected in the traditional survey not seen in the eDNA survey (Fig. 5b). In general, the eDNA survey tended to detect smaller flowers with a variety of pollination syndromes such as *Ambrosia confertiflora* and *Brickellia eupatorioides*. The forbs that were only detected by the traditional survey were typically rarer plants with large showy flowers such as *Berlandiera lyrata*. This makes sense as the large showy flowers would grab the attention of surveyors during a traditional survey, but if the species is rare, it may not contribute much eDNA to collected samples. Additionally, while both surveys detected cactus DNA, it appears that traditional survey was better at detecting multiple cacti and low activity species. It is meaningful to note that while we have identified these trends, some exceptions exist. For example, the eDNA survey detected the larger, showy thistle *Centaurea melitensis*, while the traditional survey detected *Tragopogon dubius*, which is a wind pollinated species. Additionally, the eDNA survey detected more trees than the traditional method and the BLAST results had multiple tree species' DNA for species close to our study site (Fig. 5c). This indicates that airborne eDNA could be ideal for tree identification on larger scales as well. Understanding why eDNA and traditional surveys detect different species has been a cornerstone of eDNA research (31), and this is a question that also must be addressed within the airborne eDNA community.

Our results suggest that both traditional and eDNA survey results are influenced by temporal effects such as species seasonality. For example, from the reference library comparison data (Tables 1 and 2), the traditional and eDNA survey share five of the ten most common species across the entire surveys while the September and May surveys each share just three species. Some of these differences can be attributed to the airborne eDNA survey being much easier to implement across the entire year. Each traditional survey in May and September required hundreds of volunteer hours and individuals with taxonomic expertise to identify plant species. However, the airborne eDNA survey required about three hours for a single person to collect and filter the airborne eDNA from all nine BSNE traps approximately every two weeks (approximately 66 hours for the entire year). Since we were able to sample relatively continuously throughout the year with eDNA, we detected trends in the plant community not observable by our two traditional survey snapshots. For example, the eDNA survey found that tansy mustard (*Descurainia pinnata*) was one of the most common species but was rarely seen in the traditional survey (Table 1; Table 2). This can be attributed to a large growth and bloom event that was opportunistically observed during the eDNA surveying early in the Spring, but not captured by either formal traditional survey. For a month, our study site was dominated by this species (Johnson, personal observation), but by the time the traditional survey took place in May there was little remaining to indicate this. This highlights how the nature of an airborne eDNA survey being easier to implement and sample allowed for more species to be detected on our study site.

In addition to detecting more comparable species, the eDNA survey also detected more invasive species than the traditional survey. On our study site there are three major invasive species, Russian thistle

(*Salsola tragus*), Mexican feather grass (*Nassella tenuissima*), and tree of heaven (*Ailanthus altissima*). While both the traditional and eDNA surveys found Russian thistle and Mexican feather grass, only the eDNA survey detected tree of heaven. This is meaningful because tree of heaven represents an invasive species that is in the ideal stage for eradication. The most effective time to stop an invasive species is before it becomes established, which can be challenging if traditional methods are unable to detect an invasive fast and efficiently enough (32). In our study, airborne eDNA was able to detect the tree of heaven when it was rare and not established while the traditional survey did not.

Overall, the airborne eDNA survey was able to detect more species than the traditional survey, was more efficient, and detected more invasive species. However, it has been shown that oftentimes the best way to utilize eDNA surveying is in conjunction with traditional methods due to the strengths and weaknesses of each method (8, 33). For example, 23 species were not included in the traditional surveying method due to no reference data existing for them. While these were removed from the comparison because there is no way to know if we detected those species or not, these still constitute real species detected by the traditional survey. As time goes on the number of species without reference information will continue to drop, but for the time being the restriction of sequencing data is a limitation of eDNA surveying. By combining both methods, a more well-rounded surveying tool can be established.

Other patterns

In addition to examining the number and types of species our surveys detected, this study has also shed light on the ecology of airborne eDNA, which refers to the origin, state, transport, and fate of eDNA in the environment (31). One of the most insightful aspects of our study for the understanding of airborne eDNA analysis is that we were able to sample repeatedly over the course of an entire year, which had never been done before. As a result, we observed how species signals changed throughout the year. For example, blue grama (*Bouteloua gracilis*) was the most detected species in Event 9, the fourth most detected species overall, and not in the top ten for Event 21 (Table 2). However, this species was the most common for the total survey and both sub-surveys for the traditional survey (Table 1). This can be explained by examining the ecology of blue grama, which is pollinated in the fall (Event 9) and then goes dormant in the early summer (Event 21). During this dormancy, it is not growing, flowering, or pollinating, which minimizes its production of airborne eDNA (23).

Another example of the ecology of a species impacting detection is Russian thistle (*Salsola tragus*). Russian thistle was the second most common species detected with our eDNA survey across both sampling events and the total survey (Table 2). However, this species was not within the top ten most commonly detected species in the May traditional survey and was only common in the September survey (Table 1). These patterns can be explained by examining the unique lifestyle of Russian thistle. Russian thistle grows in the spring where it flowers and forms seeds. Once the seeds are formed the plant breaks off the ground and tumbles across the landscape spreading up to 50,000 seeds and oftentimes getting caught on fence lines (34). With the plant having various life stages and moving positions, it is hard for the traditional survey to consistently find the species. However, since the species is moving so much and

releasing so much material, the eDNA metabarcoding survey was able to consistently detect large amounts of signals from this species.

We also found that the time of year greatly impacted the number of reads collected. For example, our samples from Event 9 in the fall produced 14,517 reads for reference library analysis while Event 21 in the spring produced 63,114 reads. This is most likely because in spring more species are growing and flowering while in the fall, most species are done flowering and are not biologically active. Furthermore, the trend of springtime producing more data is shown with Event 21 detecting seven more species than were found with Event 9. We can also examine the number of species that were found at each combined collection time across the entire year (Fig. 4). The number of species found on average with each event was approximately 14 and stayed between 10 and 20 species for most of the events sampled. The large dip on October 26, April 26, and May 10 can be explained by large rain events occurring near or during the time of sampling. Johnson et al. (21) found that rain appeared to have an impact on the amount of airborne eDNA that could be collected which is also shown in our results. The lowest dip on June 14 however was not caused by rain and may be the result of data being lost during the combination of the nine BSNE traps, a dilution error, or user error during the bioinformatics pipeline. Lastly, we can see that combining the nine BSNE traps into a single combined sample led to less species gathered per event. The nine BSNE dust traps were analyzed separately for events 9 and 22 and found 39 and 46 species, respectively. While combining the samples allowed us to survey over a longer period and detect species throughout the year, we would recommend if the funds were available that future projects use as many samples as possible and avoid pooling samples.

In addition to examining how reads and the number of species changed temporally, our analysis of Event 9 and Event 21 separately allows us to examine how each trap performed spatially. Across both events, the general trend was that the more northwesterly trap detected the fewest number of species, which can be explained by the fact that the wind blows in from the northwest, and any material that is carried on the wind in that corner of the study site will not be from our rangeland (Fig. 3a and 3b). The traps in the center of the rangeland for both events collected the most species, and the number of species typically increased toward the east in line with the wind. This highlights that airborne eDNA collection must consider local factors that can quickly change such as the weather.

Additionally throughout the year there were multiple species that were consistently detected regardless of their pollination syndrome (wind or insect pollinated), flowering season, or time of year. Some examples include honey mesquite, Coulter's horseweed (*Laennecia coulteri*), Russian thistle, and Johnson grass (*Sorghum halepense*) which were found throughout the combined yearlong samples. Furthermore, multiple species were detected that are primarily insect pollinated such as tansey mustard and honey mesquite which were two of the three most common species in our reference library analysis (Table 2). This confirms previous findings that airborne eDNA can be used to detect species from more than pollen in the air and is most likely detecting leaf fragments, flower fragments, and free floating DNA alongside pollen (21).

Conclusions

Overall, compared to a traditional plant community survey approach, the eDNA survey found more species, detected more invasive species, and was able to survey our study site more often due to its relatively low time and resource requirements. Thus, we believe airborne eDNA surveillance represents a valuable contribution to the toolbox for the study and management of terrestrial plant communities. Recently, there has also been evidence supporting the presence of animal eDNA in the air, indicating airborne eDNA could be applied to terrestrial animal surveying (35). The application of airborne eDNA monitoring could be applied to areas such as invasive and endangered species detection, understanding how a disturbance (fire, storm, flood, etc.) impacts a community, and tracking the change in communities over time due to climate change. In the future more research needs to be done on the ecology of airborne eDNA and expanding this method into other life forms such as animals. As we have shown, airborne eDNA represents an especially useful plant community monitoring tool that should be further explored by researchers and managers.

Methods

Study Site

Surveys were conducted on the approximately 53-hectare Texas Tech University Native Rangeland (33.60327 N, -101.9003 W). This rangeland, located within the city of Lubbock, Texas, USA, is used and maintained as a research and teaching resource by the Texas Tech Department of Natural Resource Management (Fig. 1a). This site is primarily a short-grass prairie with many native bunch grasses, forbs, and cacti. Additionally, the site has a large population of honey mesquite (*Prosopis glandulosa*) and several documented invasive species including Russian thistle (*Salsola tragus*), Mexican feather grass (*Nassella tenuissima*), and tree of heaven (*Ailanthus altissima*). In addition to the short-grass prairie habitat, the site also contains an ephemeral playa lake, which hosts several wetland plant species. A benefit of using the Texas Tech University Native Rangeland is that this study area had a list of 165 plant species that had been observed over the course of the last two decades by professors and classes that have taken place on the property.

Traditional Survey

We conducted a traditional plant community survey at two different times in the year to capture both the spring and fall flowering seasons. The first survey occurred from September 21–25, 2018, followed by a second survey on May 23rd and 24th 2019. To capture plant community diversity, cover, and abundance, we established 9 sampling locations, each consisting of three 100m transects organized as spokes around a central point as recommended by Herrick et al. (3). The spoke designs help to reduce disturbance along the transects by focusing the disturbance to the center of the site (3). The location and size of the spokes were chosen to provide the most coverage of the rangeland property while minimizing site overlap (2). Each sampling location consisted of three equidistant 100 m transects spreading out in three random equidistant directions from a central point (Fig. 1b). These three transects result in

coverage of approximately 3.1 hectares per sampling location, and a total of nine sites were set up across the rangeland in a grid formation (Fig. 1a).

At each transect within each site ($n = 27$ total transects), two separate plant community survey methods were used: a line-point intercept survey followed by a broader visual survey. The line-point intercept survey uses a pin to measure plant diversity and abundance. Starting at a randomly selected point within the first meter of each transect, a pin was dropped at 1-m intervals, and we recorded all vegetation that intercepted or touched the pin. The line-point intercept method is reliable for determining the species found along a transect. However, the goal of this survey was to find the most species possible, so a visual survey was also conducted along the length of each transect. Any species found near our transects but not captured by the line-point intercept survey were also recorded via this second survey approach.

Airborne eDNA Collection, Extraction, Amplification, and Sequencing

To collect airborne eDNA, we deployed Big Spring Number Eight (BSNE) dust traps at the center of each site ($N = 9$) for the traditional survey (Fig. 1a; 21–23). Each BSNE trap consisted of two independent triangular metal collectors approximately 0.9 and 0.4 m above the ground (Fig. 1c). Each collector included a metal sail that aligned the opening at the front of the collector into the wind to collect dust and particles at the bottom of the trap. These traps were the most efficient passive method for collecting airborne eDNA in a previous methods comparison at our study site (22). We completed the airborne eDNA survey during the same year (2018–2019) as the traditional surveys. The airborne eDNA collection was much less labor intensive than the traditional survey methodology, so we recovered eDNA from traps approximately every two weeks for a year. Specifically, the traps were deployed from June 11th 2018 until June 14th 2019, with 22 collection events in between.

To recover the airborne eDNA from a BSNE dust trap, we used approximately 1L deionized water to wash the material out of the bottom of each collector into a sterile 1-L bottle. Both collectors on each BSNE trap were combined into a single sample, with each trap contributing approximately 500ml to the overall 1-Liter sample. We transported the rinse water to the laboratory in a cooler and vacuum filtered the samples through 1 μ m Isopore membrane filters within 2 hours of collection. Filters were stored at -20° C until the DNA extractions took place. We extracted total genomic DNA using a DNeasy PowerPlant Pro DNA Isolation Kit (QIAGEN), which demonstrated high efficiency in previous airborne eDNA analyses (22). We followed the manufacturer's protocol, except we added an extra grinding step with a sterile plastic pestle and frequent vortex agitation to ensure homogenization at the beginning of the extraction process (21–23). Extracted genomic DNA was stored at -20°C until future DNA analysis. To ensure that there was no contamination throughout this process, extraction blanks were used for each extraction along with sterile gloves, containers, and forceps.

Following DNA extraction, we pooled genomic DNA from all nine BSNE traps combined for each sampling event, creating 22 combined airborne eDNA samples. We also analyzed a subsample from each of the nine BSNE traps from sampling events on October 12, 2018 (“Event 9”) and May 31, 2019 (“Event 21”)

individually to examine how the specific eDNA sampling events that were most closely paired in time with traditional plant surveys. Thus, 40 samples were analyzed overall: 22 combined samples representing the entire landscape at each airborne eDNA sampling event, and 18 samples representing each individual sampling site on two specific dates. Finally, we diluted samples by a factor of 10 with pure deionized water to limit PCR inhibition, which we have commonly observed in airborne eDNA samples at our study site (21–23).

Samples were amplified with polymerase chain reaction using a QuantStudio 3 Real-Time PCR System (ThermoFisher Scientific). We targeted the nucleic ITS2 gene because it corresponded to an abundance of available GenBank sequences relative to other plant barcoding genes (e.g., trnL, rbcL, matK). Specifically, we amplified DNA from our samples using the forward S2F and reverse ITS4 primers as described by Keller al. (36). Each 50 µl reaction contained 1X AmpliTaq Gold 360 Master Mix (ThermoFisher Scientific), 0.16 µmol/L forward and reverse primer concentrations, and 3 µl template. The thermocycling program began with an initial 95°C step for 10 min, followed by 40 cycles of 40 s at 95°C, 40 s at 49°C, and 40 s at 72°C. Each plate contained a negative control to ensure no contamination took place during experimental setup. Amplified products were visualized via electrophoresis on a 2% agarose gel stained with GelRed (Bio-Rad). Extraction blanks were examined following the same protocol.

We cleaned and extracted amplified products from the gel with the PureLink Quick Gel Extraction Kit (Thermo Fisher Scientific). Next, we prepared each library for Illumina MiSeq amplification using the QIAseq 1-Step Amplicon library kit (QIAGEN), which ligated unique barcoded adaptors onto the ends of the DNA in each sample, while also removing excess adapters, dimers, and other contaminants. Once library preparation was completed, we quantified samples with a Qubit Fluorometer (ThermoFisher Scientific), and the fragment size for each library was determined using a TapeStation System (Agilent). Libraries were then diluted to 10nM, and pooled into a single sample to maximize equal sequence depth per sample location (37). Finally, paired-end sequencing was carried out with an Illumina MiSeq (Illumina, San Diego, CA, USA). In preparation for bioinformatic analysis, reads were demultiplexed and exported as a FASTQ files.

Taxonomic identification and comparison

We analyzed sequences by first using a reference library approach then using a more specific BLAST approach to capture any species not included in our reference library. For the reference library approach, we searched GenBank for ITS2 sequences from all plant species known to occur on the Texas Tech University Native Rangeland (N = 165) and downloaded available sequences as FASTA files. Sequences were mapped against the reference data bases using SeqMan NGen (DNASTAR INC). The software automatically aligned our data, trimmed our reads, and removed low quality redundant sequences. We selected a minimum required base pair assignment of 190 base pairs to limit assignment errors and added a successful match threshold of 99% (38). In addition to taxonomic identification of each sequence, at the end of the analysis, the number of reads from each sample were recorded for each matched reference library accession number.

To identify species using a BLAST approach, demultiplexed Illumina sequences were processed using DADA2 (39). First, both the forward and the reverse sequences were filtered and trimmed based on a generated quality profile. Next, the DADA2 algorithm used a parametric error model to determine likely errors in the sequence data and filter out those errors. Paired reads were merged (12 base pair overlap and no mismatches), chimeras were removed, and amplicon sequence variants (ASV) were output. The ASVs are considered a higher resolution analogue to operational taxonomic units (OTU) and show the unique sequences found within your samples and the number of reads associated with each ASV (39). Finally, ASVs with 10 sequences or fewer were removed from our future BLAST analysis to limit sequencing and assignment errors (40). Taxonomic assignment for the remaining unique ASVs was completed using BLASTn and the NCBI nt database. Because the BLAST approach is not influenced by a priori observation of species on the landscape like the reference library approach, we required 100% identity match for taxonomic identification. If any ASVs had a 100% match to multiple species in the same genus, the ASV was assigned to the genus level (41). For ASVs that had multiple 100% genera or species matches, all were kept following the recommendation of Klymus et al. (42). The species and genus results were then examined through the United States Department of Agriculture Plants Database (43) to classify the species or genus as being either within our study site (i.e., Texas Tech Native Rangeland), local county (Lubbock County), state (Texas), country (United States), or the World. Once labeled, the results were examined and species that were classified as being within our rangeland or Lubbock County along with making ecological sense (agricultural species were not counted for example) were considered to be a positive detection from the BLAST survey. The ASVs that could detect genera were examined separately from species assignments. Lastly, the total number of species and the types of species found were compiled for both the traditional and metabarcoding methods and compared.

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not Applicable

Availability of data and materials: Sequence results are added as an appendix on this manuscript. Raw reads will be submitted to the Texas Data Repository upon acceptance of this manuscript.

Competing interests: The authors declare that they have no competing interests.

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Author's contributions: MJ designed the research, collected and analyzed the data, and wrote the manuscript. MF analyzed the data and commented on the manuscript. RC collected the data and commented on the manuscript. MB designed the research, collected and analyzed the data, commented on the manuscript, and provided funding.

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Figures

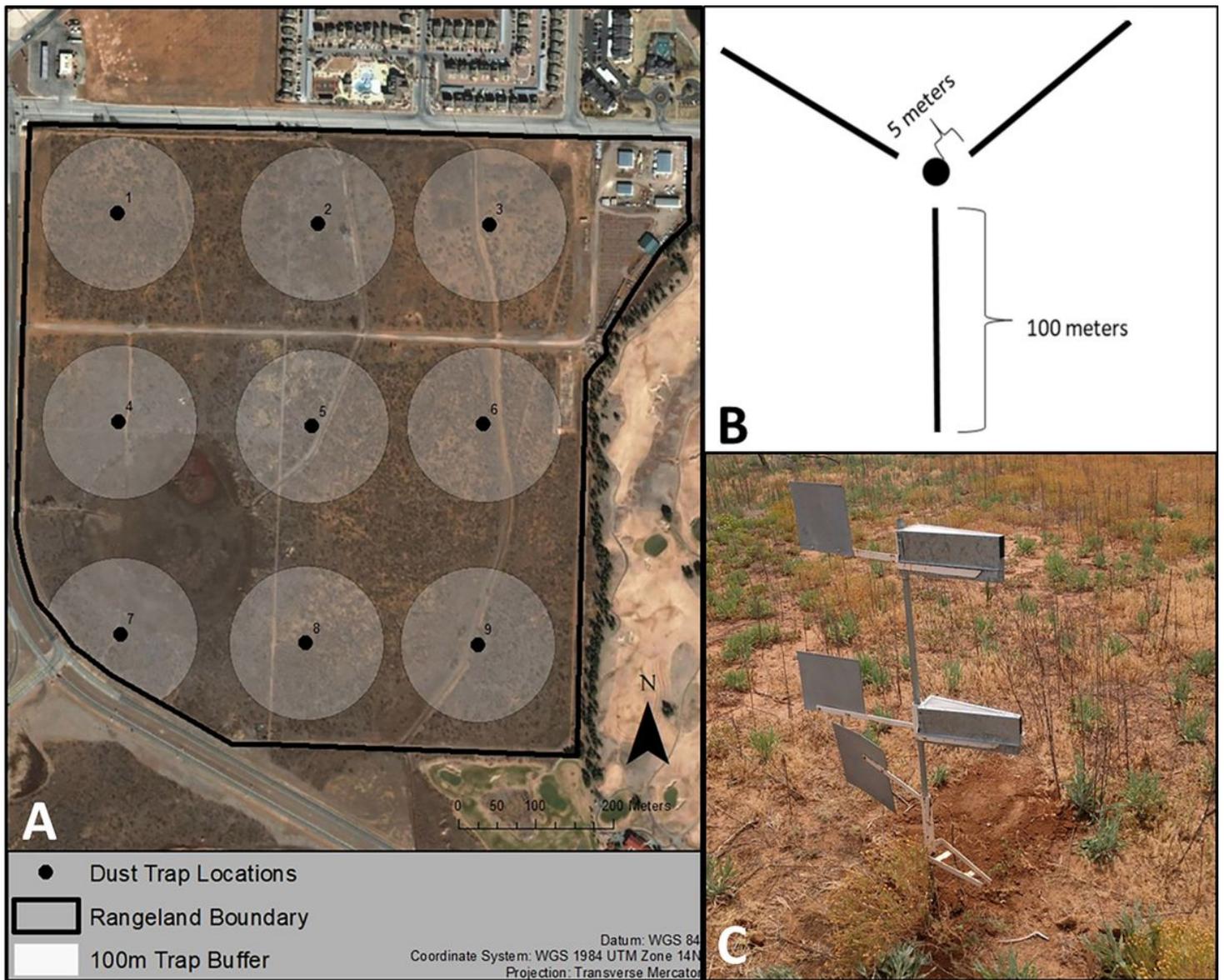


Figure 1

The Texas Tech University Native Rangeland (Lubbock County, TX, United States) study site where both the traditional and metabarcoding plant community surveys were conducted. The points and numbers represent individual BSNE dust trap locations while the buffers around each point represent the 100m traditional survey extent (A). The spoke transects used for our traditional plant community survey. Each site consisted of three 100 meter transects extending from a central point. The transects all start 5 meters from the central point to avoid overlap (B). The Big Spring Number Eight Dust traps that collected the airborne eDNA (C).

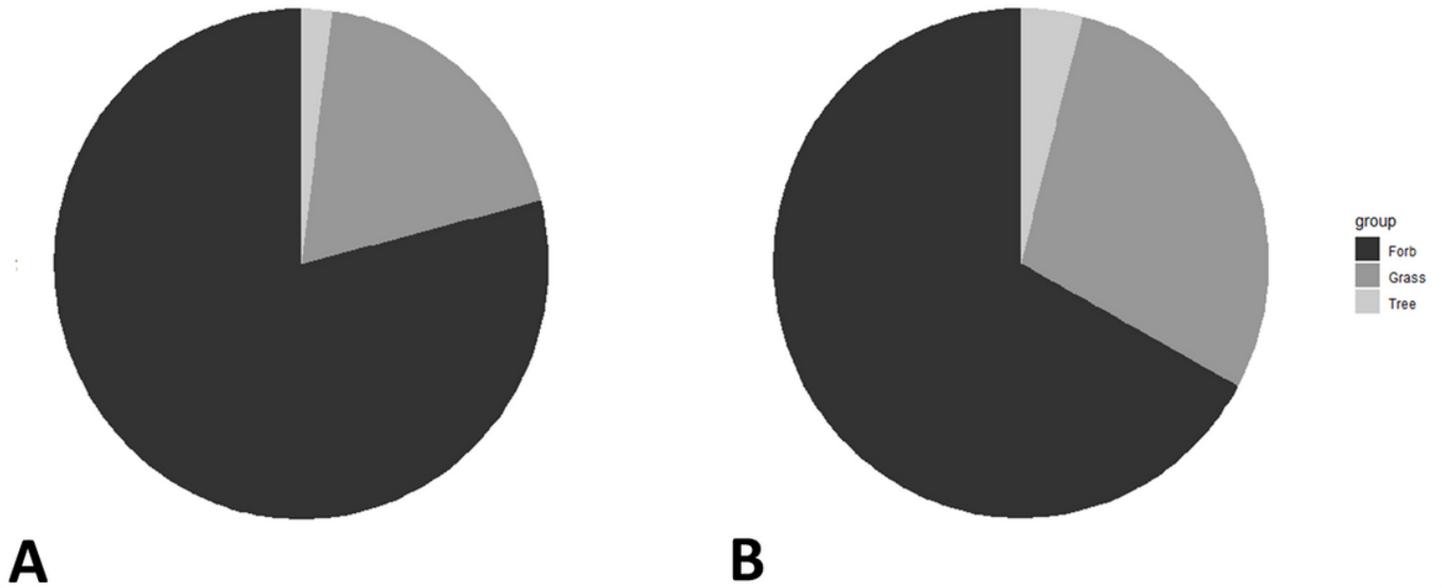


Figure 2

The percentage of forbs (79), grasses (19), and trees (2) that were found during the two traditional surveys on our study site (A). The percentage of forbs (67), forbs (29), and trees (4) found during the eDNA metabarcoding survey (B).

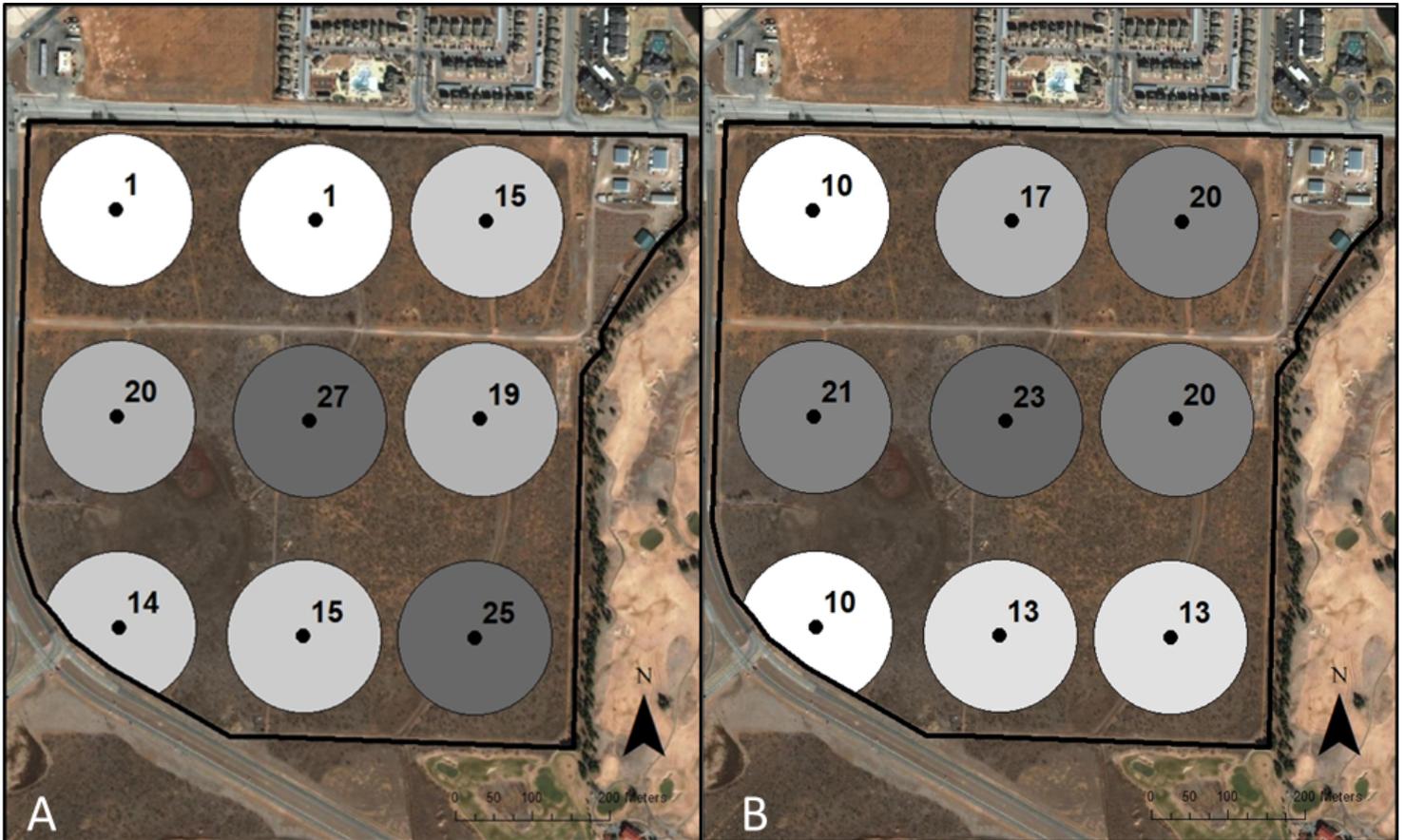


Figure 3

Figure 3a shows the number of species that each BSNE airborne eDNA trap collected from the sampling event 9 that corresponded to the September survey. Figure 3b shows the number of species that were captured by each airborne eDNA BSNE trap from the sampling event 21 corresponding to the May traditional survey. The shading represents the amount of species found by each BSNE trap, with the darker coloring indicating more species detected.

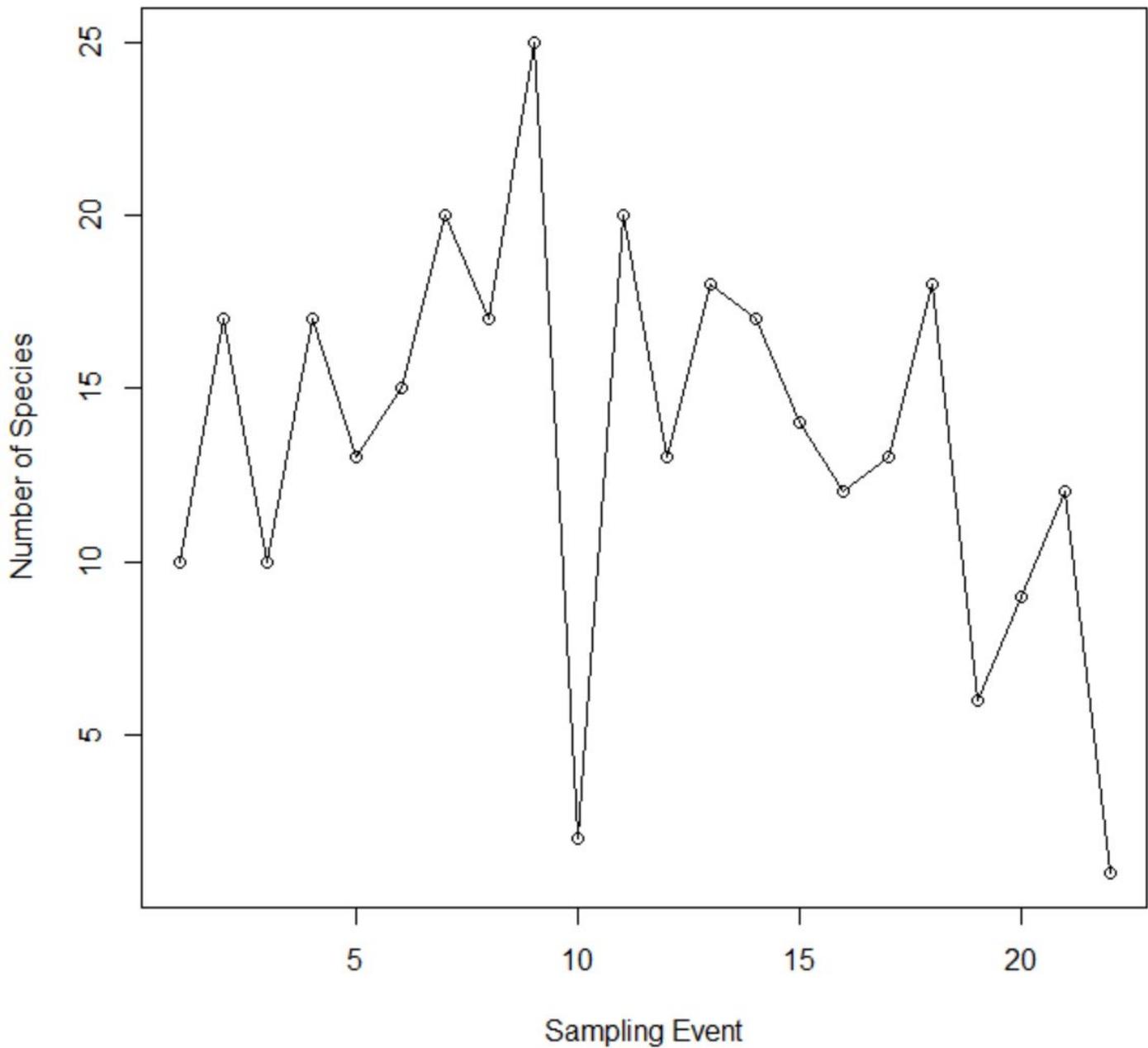


Figure 4

The number of species that were found from event 1 to event 22 during the yearlong airborne eDNA sampling.

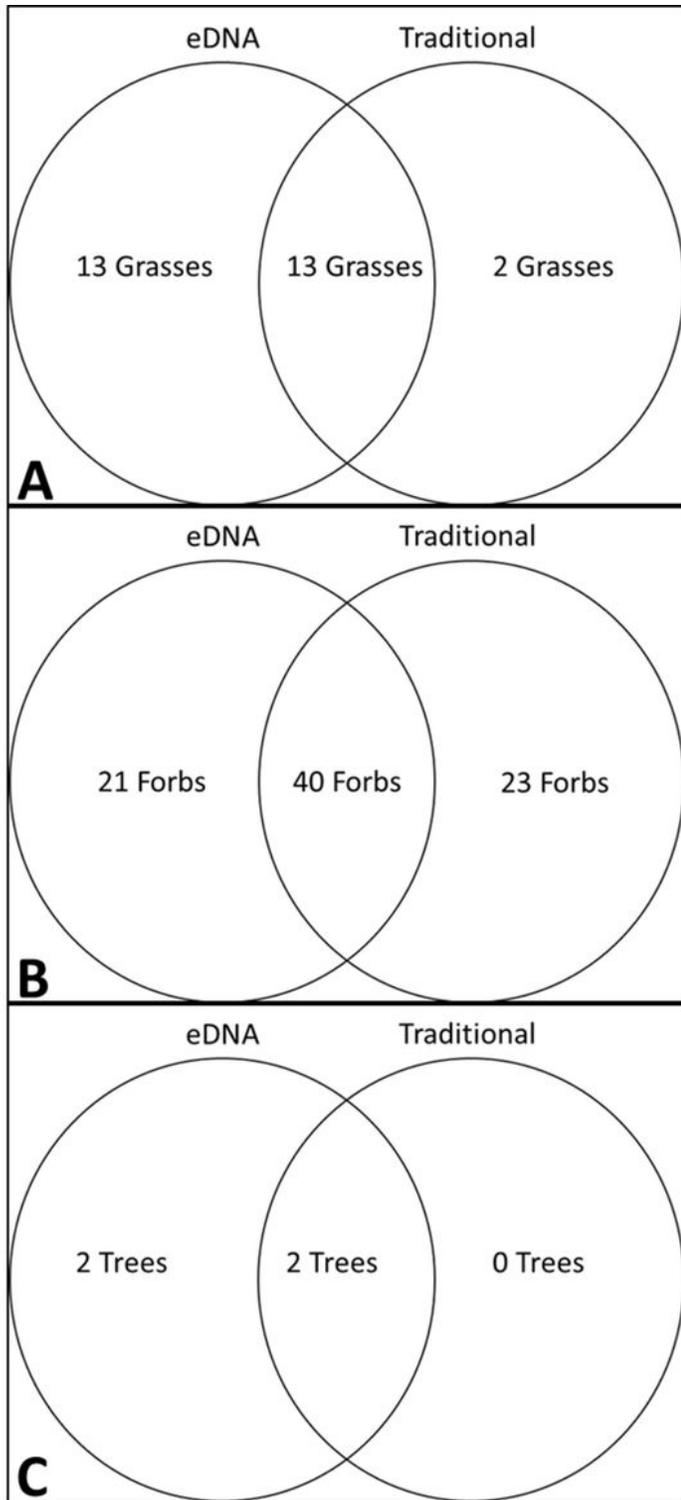


Figure 5

A Venn diagram displaying the number of grasses (A), forbs (B), and trees (C) that were found by the eDNA and traditional methods alone and together.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Appendix1.xlsx](#)