The ecology of aboveground terrestrial eDNA: Its state, transport, and fate on aboveground surfaces

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Abstract

Environmental DNA (eDNA) analysis has become a valuable tool for detecting aquatic and terrestrial species for monitoring efforts and site biodiversity assessments. However, if aboveground terrestrial eDNA surveys are to be widely adopted, it is necessary to first understand how terrestrial conditions affect the state, transport, and ultimate fate (or 'ecology') of terrestrially deposited eDNA. Many of the processes that affect the state, transport, and fate of eDNA in aquatic environments may not be applicable in aboveground systems, warranting an exploration of the terrestrial processes that likely do affect eDNA. Here we explore the ecology of aboveground terrestrial eDNA through a series of experiments evaluating the optimal filter pore size for intracellular eDNA collection, how eDNA is affected by rain events, and its degradation rate under different solar radiation conditions. We found that the captured concentration of intracellular eDNA was not significantly affected by an increase in filter pore size, suggesting there is a wide range of viable pore size options for targeting intracellular eDNA. We also found extracellular eDNA degrades more rapidly than intracellular forms when exposed to solar radiation, indicating the latter is a more viable target for collection. Finally, we identified that rainfall or mist will remove most terrestrial eDNA present on vegetation substrate. This study provides researchers and managers key insights into successfully designing and carrying out terrestrial eDNA surveys that maximize detection probability and minimize false positive results.

Introduction

Originally used to assess microbial communities of ocean sediments (Ogram, Sayler, & Barkay, 1987), the use of environmental DNA (eDNA) applications have broadened significantly in recent decades to include the detection and monitoring of a wide range of species in marine and freshwater ecosystems (Martellini, Payment, & Villemur, 2005; Ficetola, Miaud, Pompanon, & Taberlet, 2008; Jerde, Mahon, Chadderton, & Lodge, 2011; Dejean, Valentini, Miquel, & Taberlet al., 2012; Thomsen, Kielgast, et al., 2012a). The approach has also increasingly targeted terrestrial species using eDNA deposited within natural or artificial water bodies (e.g. Ushio et al., 2017; Williams, Huyvaert, Vercauteren, Davis, & Piaggio, 2018), or deposited in soils (e.g. Buxton, Groombridge, & Griffiths, 2018; Kucherenko, Herman, III, & Urakawa, 2018; Leempoel, Hebert, & Hadly, 2019; Sales et al., 2019; Walker et al., 2017). More recently, a number of novel techniques to collect eDNA deposited on substrates found in aboveground terrestrial settings (e.g. vegetation surfaces, crops, and spider webs) have broadened the application of eDNA methods to include deployment of monitoring protocols designed to survey terrestrial species and communities (Nichols, Koenigsson, Danell, & Spong, 2012; Valentin, Fonseca, Nielsen, Leskey, & Lockwood, 2018; Valentin, Fonseca, Gable, Kyle, et al., 2020; Xu, Yen, Bowman, & Turner, 2015). However, while the state, transport, and fate (i.e. the 'ecology') of eDNA in aquatic ecosystems has been thoroughly explored (Barnes and Turner, 2016) it is not well understood in terrestrial ecosystems, leaving key questions surrounding sampling design and detection rates unanswered.

Understanding the state, transport, and fate of eDNA is critical to the design and interpretation of eDNA surveys (Barnes and Turner, 2016). eDNA can be present in multiple states; either in intracellular, intraorganelle, or extracellular form (Turner, Barnes, Xu, Jones, et al., 2014). Past eDNA surveys have collected a mixture of different states through direct substrate testing (i.e. DNA extractions directly from soil, or fecal material – Kucherenko et al., 2018; Martellini, et al., 2005), or targeted specific states through chemical isolation (e.g. Minamoto, Yamanaka, Takahara, Honjo, et al., 2011; Taberlet, Prud'Homme, Campione, Roy, et al., 2012) or differential size selection via filtration or centrifugation (e.g. Turner et al., 2014; Martellini et al., 2005). Identifying the eDNA state(s) most common within the environment being surveyed, or relevant to the question being addressed, and using appropriate isolation methods to capture the desired state(s), is key to designing protocols that maximize the probability of species detection (Turner et al., 2014). Capture of specific eDNA states in suspension is typically accomplished via direct processing of water, tissue centrifugation, filtration, or a combination thereof (e.g. Martellini, et al., 2005; Goldberg, Pilliod, Arkle, & Waits, 2011; Jerde et al., 2011; Minamoto et al., 2011); with filtration being the most common approach at present. However, the existing literature guiding filtration of eDNA states via specific filter pore sizes (e.g. Turner et al., 2014; Wilcox, McKelvey, Young, Lowe, et al., 2015; Moushomi, Wilgar, Carvalho, Creer et al., 2019) does not represent the full range of pore sizes that may influence optimal capture of intracellular eDNA (i.e., trade-offs between maximum water filtration and DNA yield). Similarly, understanding how environmental conditions affect the decay of each eDNA state over time informs the interpretation of positive or negative detection results.

For instance, if a captured eDNA state persists in the environment for long periods (i.e. months or years; e.g. Andersen, Bird, Rasmussen, Haile, et al., 2012; Turner, Uy, & Everhart, 2015; Strickler, Fremier, & Goldberg, 2015) it is unknown if a positive species detection indicates a recent presence or one over a relatively long time frame. Conversely, eDNA states that degrade quickly after deposition (i.e. hours, days, or weeks; e.g. Zhu, 2006; Thomsen, Kielgast, Iversen, Wiuf, et al., 2012b; Thomsen et al., 2012a) may indicate species presence within the very recent past, or may break down beyond detectability and produce false negative survey results (Schultz & Lance, 2015). Most existing knowledge about the fate of eDNA comes from experiments conducted within water or soil, finding that biotic and abiotic factors such as pH, microbial load, temperature, and enzymatic fragmentation influence the decay rates of eDNA (Barnes and Turner, 2016; Levy-Booth et al., 2007; Nielsen, Johnsen, Bensasson, & Daffonchio, 2007). However, these biotic and abiotic factors are unlikely to determine the fate of eDNA within aboveground terrestrial systems, since eDNA in said systems likely dries shortly after deposition and is thus likely influenced predominantly, if not entirely, by solar radiation (**Figure 1**).

eDNA transport in aquatic environments is facilitated by omnidirectional diffusion, precipitation through the water column, and directional movement via currents or thermal mixing, which can redistribute eDNA meters to kilometers away from the point of original deposition (Eichmiller, Bajer, & Sorensen, 2014; Deiner, Fronhofer, Mächler, Walser, & Altermatt, 2016; Thomsen, Kielgast, et al., 2012a). These processes can increase the availability of eDNA for capture and elevate detection probability, or dilute the available eDNA beyond detectability and reduce detection probability (Schultz & Lance, 2015). Transportation of eDNA deposited within soil is not as well understood beyond recognition that eDNA is unlikely to move laterally through the soil substrate (Taberlet, Bonin, Coissac, & Zinger, 2018). Therefore, the mechanisms that influence eDNA transport in water are not applicable to aboveground terrestrial eDNA. We posit that eDNA deposited on aboveground terrestrial surfaces will be predominately transported by weather events like rainfall, transferring it to the soil where it may percolate through the soil column for unknown distances (**Figure 1**). Given the unknown nature of eDNA transport in soil, surveying for species above the soil substrate necessitates the collection of eDNA from aboveground terrestrial substrates to ensure detection. Thus, the use of terrestrial eDNA aggregation techniques, which pool eDNA from a wide geographic area into a single reservoir (Valentin et al., 2018), will become invaluable for surveys of aboveground terrestrial environments. Aggregation of aboveground terrestrial eDNA has thus far been executed in two ways: by directly collecting substrates and submersing them into a centralized container filled with solution to be sampled later (i.e. vat aggregation – Valentin et al., 2018), or actively sampling and pooling eDNA from the

substrate's surface by physically removing it (Valentin et al., 2020).

For collection of aboveground terrestrial eDNA *en masse* via aggregation from surface substrates to move into regular use across a variety of survey designs, including rare, threatened, or invasive species detection and community level assessments, further investigation of the 'ecology' of aboveground terrestrial eDNA is required. Here, we conduct a series of experiments to investigate (1) the optimal filter pore sizes for isolating extracellular aboveground terrestrial eDNA; (2) how rain events limit eDNA retention on vegetative surfaces; and (3) the rate of degradation due to time of air exposure and ultraviolet (UV) solar radiation.

Methods

Environmental DNA source material

In each experiment, we used an existing assay that targets the internal transcribed spacer subunit 1 (ITS1) of the brown marmorated stink bug (*Halyomorpha halys*), an invasive pest in many parts of its range (Valentin et al., 2018). We created a homogenous solution of *H. halys* DNA, in multiple states, (i.e. intracellular, intraorganelle, and extracellular) by collecting paper towels that lined the floor of enclosures containing *H. halys* colonies and placing these into 50 ml falcon tubes filled with double deionized water. The tube was shaken, using a 50 ml vortex adapter, for five minutes to free the excrement from the paper and suspend it in solution; hereafter called 'slurry'. The slurry was removed and evenly distributed among five 5 ml tubes, which were then stored at –20 C until used for the experiments described below. We expected freezing would result in some cells within the slurry to lyse, which would provide us with all three representative eDNA states (i.e. intracellular, intraorganellar, and extracellular eDNA) being present for our experiments. The slurry was created to remove our reliance on *H. halys* individuals, and its behavior, by allowing us to ourselves deposit eDNA for experimentation. Furthermore, given that insect cells, and more generally animal cells, are largely within the same size range (i.e. 10-20 µm, (Price & Ratcliffe, 1974; Guertin & Sabatini, 2005) it allowed us to generalize our study for a wide variety of aboveground species.

Optimal filter pore size

We determined the size distribution of intracellular eDNA by separating the states of *H. halys* eDNA from the slurry using a peristaltic pump and filter combination. Intracellular eDNA is typically targeted using filters with pore sizes in the 1–10 µm range (Turner, Barnes, Xu, Jones, et al., 2014). We thus used polycarbonate track etched (PCTE) filters with 1, 3, 5, 8, and 10 µm pore sizes to identify the optimal capture pore size of intracellular eDNA. We chose PCTE filters because the manufacturing process of these membranes results in a highly consistent pore density and uniform pore size, making inadvertent loss or capture of eDNA due to presence of other pore sizes less likely.

We carried out the experiment in parallel using twenty Nalgene 150 ml plastic vacuum filter units (Nalgene Nunc International, Rochester, NY, USA) with each filter size represented by five replicates, four for experimentation and one as a negative control. We paired each filter unit with a 100 ml glass bottle containing 80 ml of deionized water and 15 µl of slurry (no slurry was added to the negative control). After filtering, we immediately extracted intracellular DNA using the HotSHOT method, which lyses cells via incubation at 95^{o}C in sodium hydroxide for one hour (Truett et al., 2000), then stored extractions at -20 C prior to qPCR analysis. We carried out qPCR in triplicate using an Applied Biosystems StepOne Plus real-time PCR machine (Applied Biosystems, Foster City, California, United States) amplifying 96bp of the internal transcribed spacer subunit 1 (ITS1) nuclear DNA via the TaqMan assay used in Valentin *et al* . (2016; 2018). We used 20 µl reactions with 500 nanomolar (nM) concentration of each primer, 250 nM of the probe, 1X TaqMan® Environmental Master Mix 2.0, and 2 µl of DNA, following a reaction protocol with an initial denaturing step of 96°C for 10 min, followed by 40 cycles of denaturing for 15 s and annealing and extension at 60°C for 1 min. We converted the DNA quantity data to copy number and averaged values for each qPCR technical replicate by sample. We then evaluated the response of within and between filter copy number to filter pore size using two iterations of generalized linear models (glm; R v.3.6.1). First, we treated filter pore size as a categorical variable, assessing the variability within and among filter size treatment groups. We then assessed whether mean eDNA abundance was positively or negatively correlated with filter pore sizes

(in microns) by repeating the analysis and treating filter size as continuous variable.

Persistence under simulated rainfall

We carried out rainfall experiments in a controlled laboratory environment using a pressure sprayer filled with deionized water and an adjustable nozzle to alter droplet size from a direct stream to a fine mist. To simulate vegetation, we used six tomatoes and six peaches to provide a comparison between smooth and textured surfaces. Tomatoes and peaches were first washed and dried to remove any external contamination, before 3µl of slurry were deposited onto five of the six pieces of fruit, leaving one as a negative control. Once the slurry had dried, we placed four tomatoes and four peaches onto an ˜35 x 25 cm metallic mesh grate, which was first cleaned with 10% bleach solution and rinsed. The last tomato and peach were kept as positive controls. Fruit were then sprayed to simulate rainfall using a handheld pressure sprayer from one meter away with a maximum working pressure of 36 PSI (2.48 Bar) and maximum water exit speed of approximately 10 meters per second; which would result in comparable speed and kinetic energy of heavy rainfall (van Boxel, 1997; Tilg, Vejen, Hasager, & Nielsen, 2020). We began by using a large nozzle opening to represent large droplets during heavy rain and sprayed 220 ml of water over the fruit. Using nitrile gloves, all pieces of fruit were then placed alone into two-liter buckets (cleaned with 1% bleach and double rinsed) filled with a liter of deionized water to remove any eDNA remaining on their surface (Valentin et al., 2018). Fruits were then removed from the buckets and the resultant solution filtered through a 10 µm PCTE filter to capture intracellular eDNA. We then rewashed the tomatoes and peaches in a 1% bleach solution then double rinsed in deionized water and randomly assigned one of each as a negative each time to control for decontamination errors. We repeated the above process with an intermediate nozzle opening to represent light rainfall and sprayed 220 ml of water, then once again after changing the nozzle to simulate a misty rain event and spraying 220 ml of water. We repeated the mist process an additional three times, halving the volume of mist applied each time (i.e. 110 ml, 55 ml, 27 ml). We then extracted the DNA from the filters using the HotSHOT method and tested for target intracellular eDNA via qPCR in an Applied Biosystems 7500 real-time PCR machine in duplicate following the above protocol.

UV degradation

We tested UV degradation of both intracellular and extracellular eDNA in four experimental treatments: 1) full sun, unshaded solar exposure from dawn until dusk; 2) half sun, unshaded solar exposure from dawn until 1pm and in full shade thereafter; 3) full shade, shaded from solar exposure for the full day; and, 4) no sun, where samples were kept within a completely dark workstation blocking all UV (e.g. CC-150 Cabinet, Spectroline, Westbury, NY, USA) to disentangle time from UV solar radiation. The *in situ* experimental site was located in a temperate environment (40.49º latitude) during mid-summer (25–31 July 2019). The three experiments were conducted simultaneously over seven days in an empty lot in New Brunswick, New Jersey, USA, in three locations no more than twenty meters from each other to provide three treatments: full sun, half sun, and full shade. The no sun treatment was completed in the laboratory from the 5^{th} to the 11^{th} of December 2019.

The full sun treatment was carried out at the top of a 3.15 m tall tree away from other structures so as to never be shaded, the half sun treatment along a fence-line facing the southern direction, and the full-shade treatment was conducted below the canopy of mature oak trees. We used living leaves from vegetation already present as our testing surfaces for the *in situ* experiments and Parafilm (Amcor, Neenah, Wisconsin, USA) for the no sun treatment. Leaves were not used in the no sun (completely dark) treatment as they would likely have died and / or desiccated, possibly affecting results. We added 15 µl of previously unused slurry to each of 35 surfaces in each treatment to provide five daily replicates throughout the experiment. Leaves that received slurry droplets were marked with lab tape on the stem for later identification. On day zero (i.e. before placing the slurry droplets for each experiment, and not exposed to the sun) we filled four 100 ml glass bottles with 80 ml of deionized water and added 15 µl of slurry. We then filtered three intracellular and one extracellular positive controls (captured in series using a 10 µm filter first then a 0.2 µm filter) to establish baseline concentrations. This was repeated with five bottles containing 80 ml of water and 15 µl of slurry to produce five intracellular and extracellular (captured in series) positive controls for the

no sun treatment. Each day (in 24 h intervals), five samples were collected from each treatment and placed in labeled sterile 50 ml falcon tubes, in addition to a sixth leaf (or blank parafilm) that did not receive any slurry for a negative control. We collected all samples while wearing fresh nitrile gloves for each treatment and avoided the lab tape used in UV sun and shade treatments to prevent possible contamination.

We returned leaves from the *in situ* sun and shade treatments to the lab to be processed immediately after collection (**Figure2**). We added 40 ml of deionized water to each of the falcon tubes containing samples and shook them twice. We then filtered the solution from each treatment as follows: full sun was filtered in series, using first a 10 µm filter into a 0.2 µm filter, to capture intracellular and extracellular eDNA, respectively; and the remaining three treatments were filtered to collect only intracellular eDNA due to 0.2 µm filter limitations. A filter negative control was present for each treatment to ensure no contamination occurred during filtration. We then immediately extracted DNA from filter membranes using the HotSHOT method and stored extracts at -20 C until all samples had been collected and processed. We analyzed all samples via qPCR in duplicate for 40 cycles on an Applied Biosystems 7500 real-time PCR machine and converted quantified concentrations to copy number as outlined above.

We calculated the decay rate (r) from $r = 1 - e^k$ for eDNA in each treatment by fitting the following model via non-linear least squares to estimate the decay constant k (nls, R v. 3.6.1; R Core Team 2019):

$$\frac{\text{Copy number}}{10^6} = Ce^{-kX}$$

where X represented either time of collection (in hours) or cumulative UV exposure, depending on the model evaluated, and Crepresented the intercept (i.e., estimated copy number in millions when$X = 0$). Within each treatment group, we ran competing models estimating the rate of eDNA degradation between cumulative UV-A, UV-B, and UV-A+B exposure, as well as time continuous decay. UV exposure data were obtained from the USDA UV-B Monitoring and Research Program for Geneva, New York, Beltsville, Maryland, and Queenstown, Maryland. We used the extrapolation tool in ArcGIS version 10.7.1 to estimate daily UV totals for UV-A and UV-B over the course of our experiment. As cumulative UV exposure increases with time, we expected the curves for time and UV to show similar patterns. We evaluated the models using AICc and judged them to be similar in performance if within 2 AICc units. Thresholds were placed at 39 PCR cycles (0.0015 ng or 1.45 $\times 10^7$ copies) to provide a conservative limit of detection, and 40 PCR cycles (0.00075 ng or 7.24 $\times 10^6$ copies) for the absolute limit of detection for our qPCR runs. The time to reach each threshold copy number was calculated from the decay curves using the following equation:

$$Time\ to\ threshold = \ln\left(\frac{\text{threshold}}{C \times 10^6}\right)\ \left(-\frac{1}{k}\right)$$

Results

Optimal filter pore size

We found 1 µm PCTE filters had the highest number of intracellular eDNA copies (mean: 970,000), with the mean number of copies descending to 937,000 for 3 µm, 801,000 for 8 µm, 762,000 for 5 µm, and the fewest copies detected with 10 µm (596,000). We found the 95% confidence intervals of all but the 10 µm filters overlapped the zero line when assessing the deviation from global mean (**Figure 3**), and there was no significant difference in copy number among different pore size categories using averaged data from qPCR technical replicates ($F_{4,15} = 0.34$, p = 0.84). We found a non-significant decrease of ˜37,500 copies per µm increase in filter size when we treated filter pore size as a continuous variable (beta = 37,479, 95% CI = [-105,214, 30,257]; $p = 0.26$).

Persistence under simulated rainfall

We found 220 ml of simulated rainfall, irrespective of whether it was heavy, light, or a mist, produced no positive eDNA detections across all eight qPCR replicates (**Table 1**). Reducing the volume of mist to

110 ml resulted in a single positive qPCR replicate on tomatoes, while peaches produced four out of eight positive qPCR replicates over four sample replicates (**Table 1**). We found 55 ml on tomatoes produced two qPCR positives over a single sample replicate, and peaches produced three qPCR positives over two sample replicates. We found the results for tomatoes were unchanged with 27 ml of water, whereas peaches produced one qPCR positive (**Table 1**).

UV degradation

Although a heavy rainstorm terminated the UV experiment at day 6, we found extracellular eDNA in our full sun treatment was not detectable beyond day four, with very few detections within the first four days of sampling (**Table 2**). For the full sun and half sun conditions, we found intracellular eDNA was detectable throughout all six days of sampling, with full shade only detectable up to day five (**Table 2**). DNA from our no sun treatment was detectable across all six days, with all samples and qPCR replicates providing positive results (**Table 2**). Our non-linear least squares models of copy number decay performed similarly based on AICc, whether the independent variable was cumulative UV exposure or time elapsed for both intracellular and extracellular eDNA (**Table 3**). We estimated that extracellular eDNA, under the full sun treatment, had a decay rate of 18.7% per hour and was detectable for 22.7 and 26.8 hours for the two thresholds, respectively (**Figure 4a**), and intracellular eDNA had a decay rate of 3.0% per hour and was detectable for 88.1 and 111.6 hours, respectively (**Figure 4b, Table 4**). The decay rate of eDNA for our half sun treatment was 2.1% per hour and was detectable up 118.3 and 152.2 hours, respectively, and our full shade treatment had a decay rate of 1.6% per hour and was detectable for 143.3 and 186.6 hours, respectively (**Figure4b, Table 4**). We found no evidence of a significant decrease in copy number for intracellular or extracellular eDNA over the 144 hour experiment in our no sun treatment (**Table 4** , **Figure5**), indicating time alone did not contribute to the decay of eDNA across UV treatments and our observed eDNA degradation was likely due to cumulative UV exposure.

Discussion

Dynamics of the state, transport, and fate of eDNA can have serious implications for determining species presence in molecular detection studies and can result in false positive or false negative results (Darling and Mahon, 2011; Schultz & Lance, 2015). Such errors can have unwelcome consequences when management actions are implemented or withheld as a result (Darling and Mahon, 2011). For example, failing to detect a nascent invasive species' population may delay rapid response and eradication efforts, or failure to detect the presence of a rare native species may result in a failure to implement conservation measures. If eDNA is to be utilized to detect terrestrial species, the ecology of terrestrially deposited aboveground eDNA must be carefully documented so that sampling and survey methods maximize detection probability and avoid false positive results. To this end, we explored the ecology of aboveground terrestrial eDNA, which is elsewhere poorly documented. To accomplish this, we made a slurry from *Halyomorpha halys* excrement to allow us to ourselves deposit eDNA on surfaces for experimentation. In doing so, our results were not contingent on *H. halys* specimens in the field or its behavior, as its only contribution to the study was eDNA in various states (i.e. intracellular, intraorganellar, and extracellular). We found no significant difference in the amount of intracellular eDNA collected among filter pore sizes from 1 to 10 μm, and while extracellular eDNA may be collected with 0.2 μm filters, its rapid degradation from aboveground surface substrates make it an unreliable source of eDNA to determine species presence. Furthermore, rainfall has a dramatic influence on the persistence of aboveground terrestrial eDNA as even mild rainfall will remove most eDNA that otherwise could have been available for collection. These results provide three critical insights for using eDNA in surveys of aboveground terrestrial ecosystems.

First, while we did not see a significant difference between filter pore sizes in our experiments, there was a trend towards an inverse relationship between eDNA copy number and filter pore size, potentially due to greater capture of free-floating nuclei in addition to intact cells (insect nuclei are ˜4–10 μm in diameter; Price & Ratcliffe, 1974). The increased capture of nuclei (or mitochondria if the target is a mtDNA locus) could extend the detectability window of this source of DNA since the persistence of organelles within the environment would be in addition to the persistence time of intact cells. This outcome may or may not be

desirable if the goal of the survey is to detect species that were recently present within a terrestrial ecosystem. There is however a more practical consideration to the choice of pore size for sampling terrestrial species. Under field conditions, smaller pore sizes restrict the volume of solution that can be filtered before filter saturation, which likely decreases the sensitivity of the survey or requires a greater number of samples (and expense) to achieve similar levels of detection (Schultz & Lance, 2015). Valentin et al. (2018, 2020) found that 10 μm filters were more practical for field sampling of aboveground terrestrial eDNA within agricultural and forested ecosystems than smaller pore sizes, as greater volumes of solution could be filtered before filter saturation occurred. Our results echo Turner et al. (2014) in that the choice of filter pore size will reflect a trade-off between the eDNA yield per volume filtered and total filtering capacity, which can affect detection of rare species (Schultz & Lance, 2015). Given that animal cells generally average 10-20 μm in diameter (Price & Ratcliffe, 1974; Guertin & Sabatini, 2005), our results should be highly generalizable to numerous invertebrate and vertebrate species of interest.

Second, our results suggest that while collecting aboveground terrestrial eDNA, emphasis should be placed on intracellular rather than extracellular eDNA due to the extremely short retention time of extracellular eDNA in terrestrial settings. Interestingly, this result ran counter to our initial expectation. We assumed the fragment size of our target DNA region (i.e., 96 bp of ITS1 nuclear DNA) was sufficiently small that it would be retained within the environment for an exceedingly long time, much like in soils (Andersen et al., 2012), thus being a concern when estimating rates of false positives (i.e. for recent presence) during eDNA surveys. Rather, the brief persistence of extracellular eDNA within aboveground terrestrial environments means it is likely to be sufficiently degraded prior to its collection from the field. However, high-frequency surveys targeting extracellular eDNA may be possible for discerning occupancy of a species when fine temporal grain sampling is the survey objective (surveys that track occupancy over days or weeks). Ultimately, the specific research objective will dictate whether intracellular or extracellular eDNA is the more suitable target DNA state.

The decay rates of eDNA we document may be accelerated or decelerated depending on seasonality and location. Our study was conducted in mid-summer at temperate latitude (40.49° latitude), which dictated UV exposure over the course of a day post-deposition. Had these experiments been conducted in a more equatorial region, then eDNA may have decayed more rapidly due to elevated solar radiation levels. Perhaps more interestingly, we observed substantial degradation of intracellular eDNA in full shade, which we assumed would have resulted in a slower decay rate than what we observed. This result could be due to reflected UV (*i.e.* , albedo) (Lenoble, 2000) indicating that environments with a high albedo may see decay rates closer to full solar exposure regardless of whether they are shaded (J. Turner & Parisi, 2018). Environments with very high albedo (i.e. snow-covered environments; J. Turner & Parisi, 2018) may see higher than expected degradation rates if estimating decay solely from UV exposure, or assuming low levels of UV exposure due to latitudinal position and/or weather, and not accounting for albedo. While we did not record temperature data, there remains a question of how temperature interacts with UV to determine terrestrial aboveground eDNA decay rates. Several studies have documented the effect of temperature on eDNA decay, though to our knowledge all such studies took place within aquatic environments (Eichmiller, Best, & Sorensen, 2016; Jo, Murakami, Yamamoto, Masuda, et al., 2019; Tsuji, Ushio, Sakurai, Minamoto, et al., 2017; Strickler et al., 2015). eDNA within aquatic ecosystems is largely suspected to degrade from microbial and enzymatic activity (Eichmiller et al., 2016; Jo et al., 2019; Tsuji et al., 2017; Strickler et al., 2015), which is unlikely to be as dominant a degradation force in aboveground terrestrial ecosystems as desiccation of cells is rapid. Nonetheless, differences in ambient temperature and humidity are a clear next step in understanding the ecology of aboveground terrestrial eDNA, especially as they interact with UV exposure. Seasonal and locational differences in UV exposure deserve further study as they will affect decisions for deployment such as the sampling window and the frequency of site visitations to carry out terrestrial eDNA surveys.

Finally, even a small amount of rain or mist drastically reduces the quantity of eDNA present on aboveground terrestrial surfaces. Without accounting for weather preceding an eDNA survey, the results of such surveys will likely produce an abundance of false negative results. We found eDNA was better retained on textured

vegetation surfaces over smooth ones during mild weather conditions (i.e. misty rain). Yet, no matter what the intensity of rain, so long as a sufficient quantity fell (220 ml in our trials), a near-complete removal of eDNA results. This result is corroborated by the findings of Staley et al. (2018), who found that eDNA derived from aboveground terrestrial species can be sampled within nearby waterways right after heavy rainfall events. eDNA removed from surfaces due to rain will thus also make its way into the soil, which can then be used to collect eDNA derived from aboveground terrestrial species (e.g. Buxton et al., 2018; Kucherenko et al., 2018; Leempoel et al., 2019; Sales et al., 2019; Walker et al., 2017; Staley, Chuong, Hill, Grabuski, et al., 2018). However, the life cycle of eDNA as it moves from aboveground vegetation into the soil column, and even deep into the subsoil (Andersen et al. 2012), remains poorly understood and warrants further exploration of the transport of eDNA through the soil column.

Here we report novel insights into the ecology of aboveground terrestrial eDNA and highlight several dynamics that are key to designing and deploying a terrestrial eDNA survey. By better understanding these processes, surveyors can account for environmental influences, such as rainfall and UV on detection dynamics, to develop best practice approaches that mitigate erroneous results from terrestrial eDNA surveys. When combined with laboratory best practices, like multi-level controls (Harper, Buxton, Reese, Bruce et al., 2019), such efforts allow for the development of robust survey frameworks for species-specific and community-level terrestrial eDNA surveys.

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Data Accessibility

Additional data files for all experiments carried out throughout within this manuscript were submitted to Open Science Framework (https://osf.io/dashboard), doi:10.17605/OSF.IO/8GK7Q.

Author Contributions

REV carried out and oversaw the field and laboratory experiments, assisted with and oversaw the analyses, and wrote the manuscript. MCA and DJW conducted the analyses and wrote the manuscript. KEK carried out the field and laboratory experiments. JLL provided funding and resources, oversaw the project, and wrote the manuscript.

Table 1 : Rain experiment showing positive or negative eDNA detection on tomatoes and peaches. Boxes in white indicate results of sample replicates (i.e. individual fruits tested), and boxes in grey indicate results of the total qPCR replicates (two replicates per sample).

	Tomatoes (smooth surface)	Tomatoes (smooth surface)	Peaches (textured surface)	Peaches (tex
	Positive	Negative	Positive	Negative
Heavy rain (220ml)	0	4	0	4
	0	8	0	8
Light rain (220ml)	0	4	0	4
	0	8	0	8
Mist (220ml)	0	4	0	4
	0	8	0	8
Mist (110ml)	1	3	4	0
	1	7	4	4
Mist (55ml)	1	3	2	2
	2	6	3	5
Mist (27ml)	1	3	1	3
	2	6	1	7

Table 2 : Detection data of terrestrial eDNA degradation under UV solar radiation treatments and no sun control treatment. "Extra" signifies extracellular eDNA and "Intra" signifies intracellular eDNA. "Pos." indicates the number of samples that were found to be positive for eDNA, and "Neg." indicates the number of samples that were found to be negative for eDNA.

	Extra (full sun)	Extra (full sun)	Intra (full sun)	Intra (full sun)	Intra (half sun)	Intra (half sun)	Intra (
	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.
Day 1	2	3	5	0	5	0	3
Day 2	1	4	4	1	4	1	5
Day 3	0	5	1	4	3	2	3
Day 4	2	3	3	2	2	3	5
Day 5	0	5	3	2	3	2	2
Day 6	0	5	1	4	2	3	0

Table 3 : Model comparisons (AICc) for cumulative UV and time decay models for each of the degradation treatments.

	Time	UV-A	UV-B	UV-A + B
Extracellular (full sun)	245.75	245.79	245.78	245.79
Intracellular (full sun)	352.49	352.35	352.42	352.35
Intracellular (half sun)	340.80	341.24	341.16	341.24
Intracellular (full shade)	321.34	321.85	321.70	321.84

Table 4 : Estimated terrestrial eDNA decay rates and maximum detection thresholds.

	Decay rate (per hour)	Upper Threshold (hours)	Lower Threshold (hours)
Extracellular (full sun)	0.187	22.7	26.8
Intracellular (full sun)	0.030	88.1	111.6
Intracellular (half sun)	0.021	118.3	152.2
Intracellular (full shade)	0.016	143.3	186.6
Intracellular (no sun)	˜0	NA	NA
Extracellular (no sun)	˜0	NA	NA

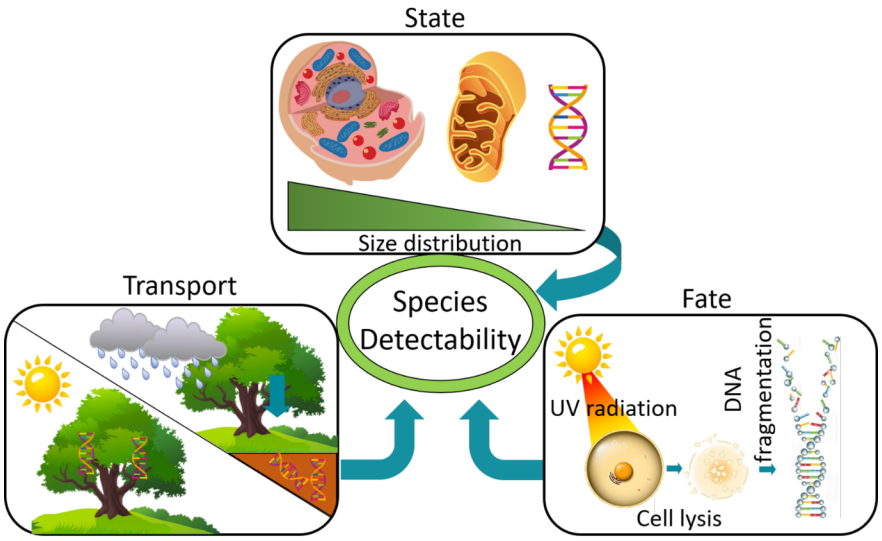

Figure 1 : Species detection using eDNA deposited on vegetation surfaces is contingent on several factors. First, an appropriate eDNA state must be selected for the question being addressed, as well as the correct filter pore size to effectively collect said state. Second, the transport of eDNA must be taken into consideration when preparing a sampling protocol. Rainfall will be the predominant influence facilitating the transport of eDNA from the vegetation surface onto another substrate or, more likely, into the soil below. Third, the fate of eDNA will affect detection, as abiotic factors such as solar radiation (UV) will result in cells and organelles being destroyed (i.e. cell lysis), followed by fragmentation of extracellular eDNA.

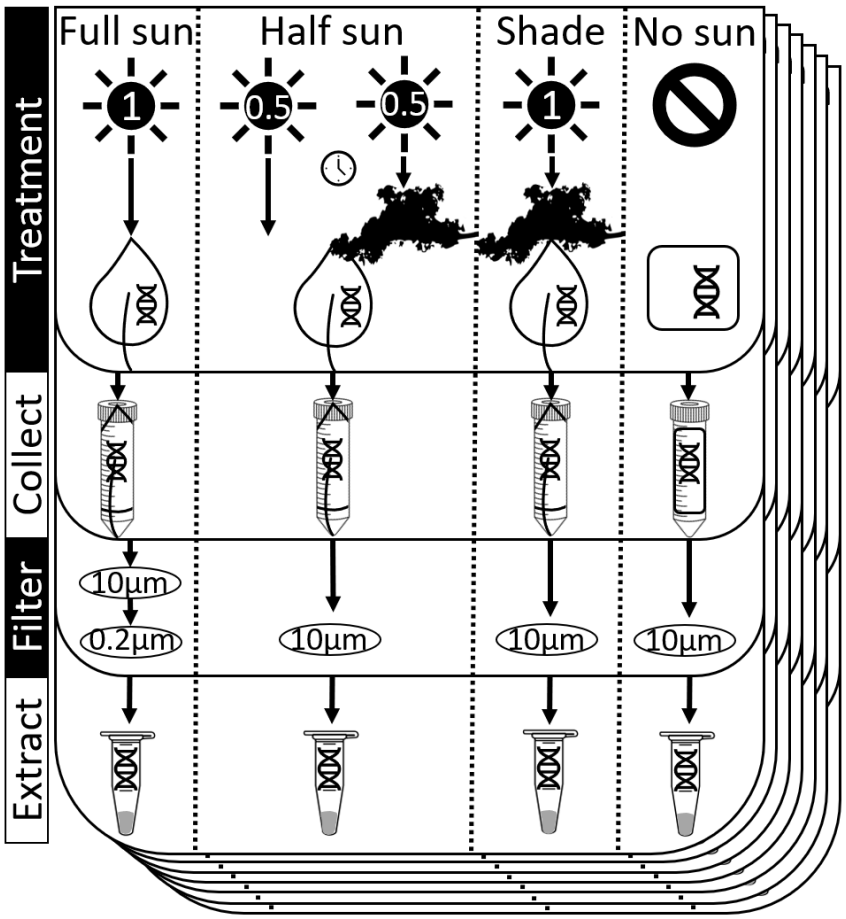

Figure 2: Workflow for UV degradation experiments. Each layer represents a different day of sampling, with each day containing four experimental treatments: full sun, half sun, shade (i.e. full shade), and no sun. The numbers within the sun for the first three treatments indicate the proportion of each day eDNA was exposed to the indicated conditions. Samples from each treatment are collected and filtered each day, then the DNA is extracted from filters and stored until all days have been completed.

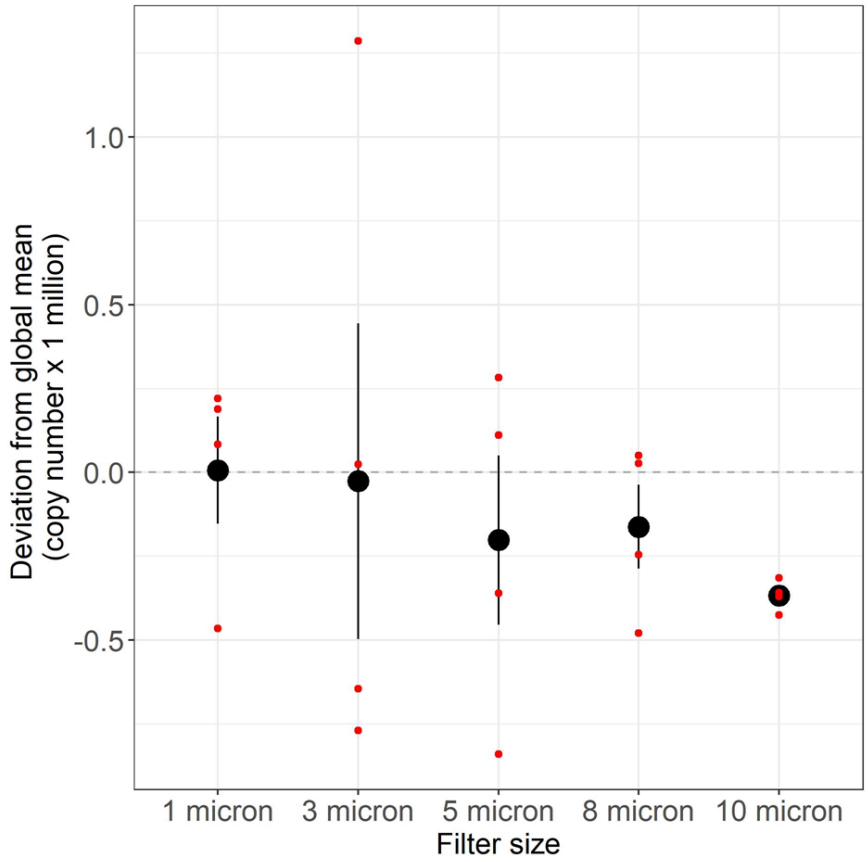

Figure 3 : Deviation from global mean DNA copy number recovered in samples, categorized by filter pore size. Red dots represent mean eDNA abundance (in copy numbers) for each sample replicate, and the black dot the mean (± 1 SE) deviation from the global mean (represented by the dashed zero line).

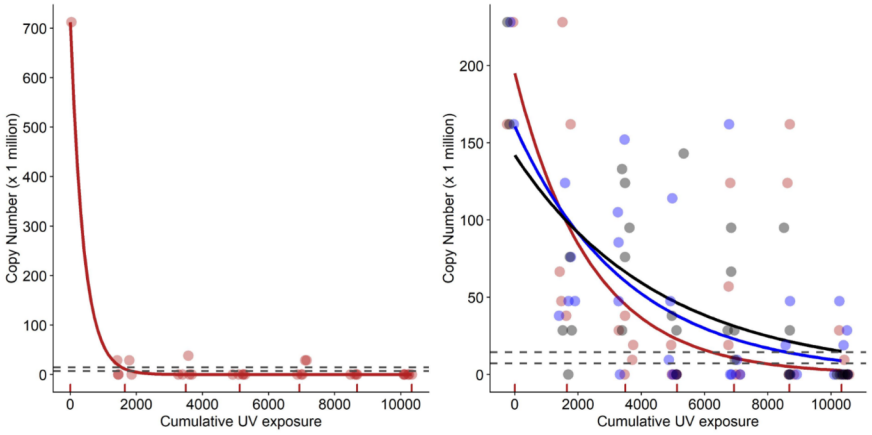

Figure 4 : Degradation curves of a. extracellular eDNA and b. intracellular eDNA under UV solar radiation treatments, as determined from non-linear least squares. In b. the red line represents the full-sun treatment, the black line the half-sun treatment, and the blue line the full-shade treatment. In both a. and b. the

red tick marks on the x-axis represent the 24-hour intervals used throughout the experiment. Cumulative UV represents the total UV radiation from both UV-A and UV-B. Dashed lines represent the set detection thresholds, with the upper threshold set to 1.45 x 10^7 copies and the lower threshold set to 7.24 x 10^6 copies. A small amount of noise ('jittering') was added to the x-coordinate of the data points to improve visibility.

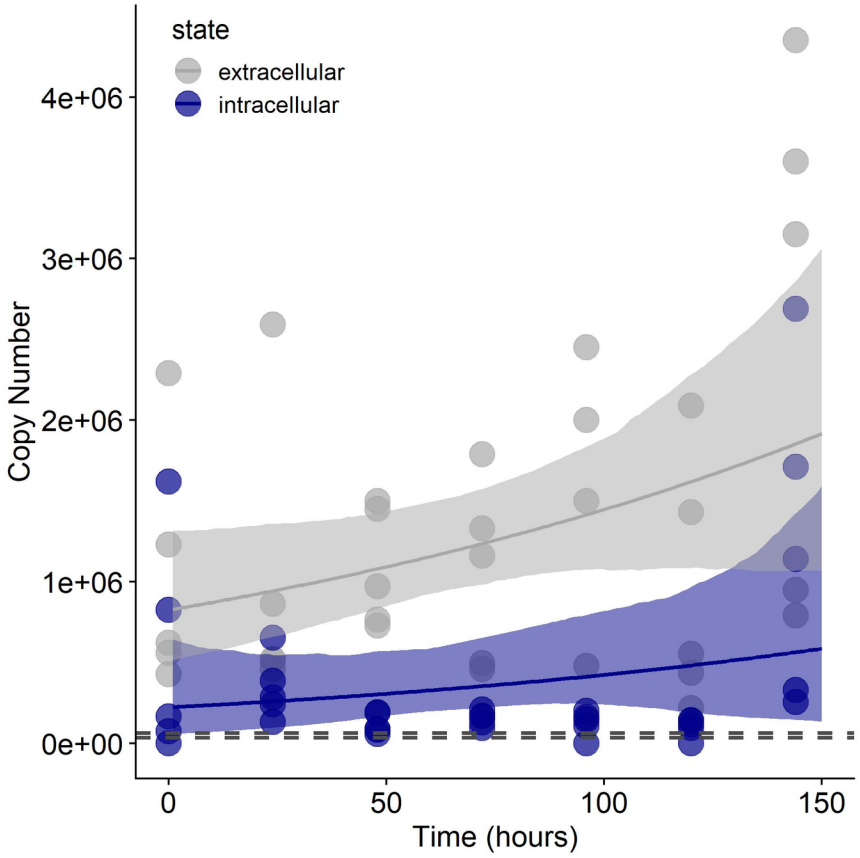

Figure 5 : Copy number measured on 35 parafilm slips spiked with brown-marmorated stinkbug fecal slurry and kept indoors, in darkness, for one of seven time intervals (from 0-144 hours with 5 replicates per time interval). Samples were suspended in water and separated into intracellular and extracellular DNA via serial filtration (10 and 0.2 μm filters, respectively). The curves represent Poisson Generalized Linear Models (GLM; log link) with non-parametric bootstrapped 95% confidence intervals. The 95% confidence intervals for the slope parameters of these lines overlapped zero (intracellular: 0.00647 [-0.00949, 0.02243]; extracellular: 0.00562 [-0.00025, 0.01150]) indicating a lack of degradation over the course of the experiment. Decay curves were not fit with non-linear least squares as with the outdoor experiments due to problems achieving model convergence. The upper and lower dashed lines below the points indicate detection thresholds representing 39 and 40 PCR cycles, respectively.