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Ground level environmental protein concentrations in various ecuadorian environments: Potential uses of aerosolized protein for ecological research

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ABSTRACT

Large quantities of free protein in the environment and other bioaerosols are ubiquitous throughout terrestrial ground level environments and may be integrative indicators of ecosystem status. Samples of ground level bioaerosols were collected from various ecosystems throughout Ecuador, including pristine humid tropical forest (pristine), highly altered secondary humid tropical forest (highly altered), secondary transitional very humid forest (regrowth transitional), and suburban dry montane deforested (suburban deforested). The results explored the sensitivity of localized aerosol protein concentrations to spatial and temporal variations within ecosystems, and their value for assessing environmental change. Ecosystem specific variations in environmental protein concentrations were observed: pristine $0.32 \pm 0.09\ \mu g/m^3$, highly altered $0.07 \pm 0.05\ \mu g/m^3$, regrowth transitional $0.17 \pm 0.06\ \mu g/m^3$, and suburban deforested $0.09 \pm 0.04\ \mu g/m^3$. Additionally, comparisons of intra-environmental differences in seasonal/daily weather (dry season $0.08 \pm 0.03\ \mu g/m^3$ and wet season $0.10 \pm 0.04\ \mu g/m^3$), environmental fragmentation (buffered $0.19 \pm 0.06\ \mu g/m^3$ and edge $0.15 \pm 0.06\ \mu g/m^3$), and sampling height (ground level $0.32 \pm 0.09\ \mu g/m^3$ and 10 m $0.24 \pm 0.04\ \mu g/m^3$) demonstrated the sensitivity of protein concentrations to environmental conditions. Local protein concentrations in altered environments correlated well with satellite-based spectral indices describing vegetation productivity: normalized difference vegetation index (NDVI) ($r^2 = 0.801$), net primary production (NPP) ($r^2 = 0.827$), leaf area index (LAI) ($r^2 = 0.410$). Moreover, protein concentrations distinguished the pristine site, which was not differentiated in spectral indices, potentially due to spectral saturation typical of highly vegetated environments. Bioaerosol concentrations represent an inexpensive method to increase understanding of environmental changes, especially in densely vegetated ecosystems with high canopies or in areas needing high spatial and temporal resolution. Further research to expand understanding of the applicability of bioaerosol concentrations for environmental monitoring is supported by this pilot study.

1. Introduction

The ability to remotely and non-invasively measure indicators of environmental and biological change have been a driving force in ecological research over the past decade from satellite imagery of deforestation to non-invasive DNA testing of various animal populations using shed materials (Caruana, 2011; Garshellis, 2006; Jha and Bawa, 2006; Jiang et al., 2014; Mucci and Randi, 2007; Vina et al., 2004). A promising remotely-collected integrative ecosystem indicator is provided by aerosolized proteins, or bioaerosols (Castillo et al., 2012; Huffman et al., 2012; Pauliquevis et al., 2012; Santarpia et al., 2013). It has been recognized that ambient

Abbreviations: NPP, net primary production; NDVI, normalized difference vegetation index; LAI, leaf area index; TSP, total suspended particulates.

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air is composed of a "protein soup" containing bacteria, viruses, spores, pollen, and a slew of biological debris from humans, animals, insects, and plants all ranging in size from a few nanometers to roughly 100 microns. To date most research examining bioaerosols has concentrated on either indoor environments or atmospheric studies (Kang et al., 2012; Schneider et al., 2011; Staton et al., 2013). These studies have focused on determining local air quality, possible pathogenic transmission of aerobacteria and viruses, bioaerosol output of particular locations such as a trash dump, or the effects of large scale fires (Alvarez et al., 1995; Costa et al., 2012; Menetrez et al., 2009; Rogers et al., 1991).

Atmospheric protein studies have confirmed the existence of large quantities of aerosolized material of biological origins with as much as 56 Tg/year (>1 μm in size); however, there have been few to no studies investigating localized ground concentrations of bioaerosols or how these concentrations vary among ecosystems (Baars et al., 2012; Despres et al., 2012; Jaenicke, 2005; Rizzo et al., 2010, 2013). Besides harboring information detailing their origin through DNA and protein profiling, the sheer amount of bioaerosol material in the air could potentially provide an index of biological activity in the area. It is already known that large protein fragments shed as sloughed skin, feathers, and shells can be used as sources of DNA, amino acids, and protein profiles to investigate a single population or species (Boulanger et al., 2008; Hansen et al., 2008; Hogan et al., 2008; Kelly et al., 2014; Valle et al., 2009; Waits and Paetkau, 2005). However, broad-scale use of bioaerosols to characterize geographic areas and detect environmental change has been largely unexplored.

The aim of this study was to collect and survey total suspended particulates (TSP) samples from diverse environments throughout Ecuador representing ranges of ecosystem productivity and human alteration, in order to evaluate the amount of bioaerosol material, the relationship of bioaerosol concentration to the environment of origin, as well as specific intra-environmental comparisons. Intra-environmental comparisons described bioaerosol response to variations in seasonal and daily weather, forest fragmentation, and sampling height. Bioaerosol concentrations were then compared to several satellite-based spectral indices to determine their agreement with other indicators of ecosystem productivity.

2. Material and methods

2.1. Study areas

Samples were collected in a pristine humid tropical forest (pristine), highly altered secondary humid tropical forest (highly altered), secondary transitional very humid forest (regrowth transitional), and a suburban dry montane forest that has been mostly deforested (suburban deforested) (Table 1 and Fig. 1). Secondary forests are differentiated by the relative alteration due to human activities including deforestation activity, agriculture, and human habitation. All samples were collected during 2009, which happened to be a drought year when annual precipitation at Quito was 40% of the 100-year average. Variations in the number of samples collected per location and sampling condition reflect the availability of the testing sites and equipment reliability.

The pristine site was in a humid tropical rainforest located in the Tiputini Biodiversity Station sponsored by the Universidad San Francisco de Quito at 229 m elevation. The reserve is located on the Tiputini River, a tributary of the Amazon River, and is directly across the river from Yasuní National Park. The site is in the far eastern portion of Ecuador with minimal, mostly indigenous human population, and is currently mostly insulated from petroleum extraction and logging. Sampling occurred in mid-October 2009 and was conducted at two heights: 0.76 m, considered ground level, and 10 m, the greatest height that could be safely sampled. The typical canopy height at Tiputini was 45 m. Wildlife activity was observed near the sample site, including squirrel (*Saimiri sciureus)* and woolly (*Lagothrix poeppigii)* monkeys within 5–15 m while spider monkeys (*Ateles)* and golden mantled tamarins (*Saguinus tripartitus)* were within 25–50 m. The animals did not appear to be disturbed by the sound of sampling equipment suggesting minimal noise pollution and minimal sample bias toward lower values due to vertebrates or insects avoiding the area.

The regrowth transitional site is near Tena, located on the eastern slope of the Andes Mountains facing the Amazon Basin at 445 m. Samples were collected on the grounds of the Andes and Amazon Field School located on the Napo River. This area lies between basin rainforest and cloud forests at higher elevations. At the time of collection the area was not agriculturally active, and

Table 1
Ecuadorian sampled location names and site descriptions.

Location name	Description	Latitude/longitude	Elevation (m)	Annual precipitation (mm)	Samples
Secondary transitional very humid forest-buffered (Tena)	Eastern slope Andes, buffered by a kilometer of forest	S 01°02′36.7″ W 077°43′05.0″	445	4500–5000	5
Secondary transitional very humid forest-edge (Tena)	Eastern slope Andes, constrained physically within 25 m	S 01°02′21.8″ W 077°43′09.0″	445	4500–5000	6
Highly altered secondary humid tropical forest (Ipatoá)	Western slope Andes, has significant agricultural activity and within 25 m of a road	N 00°07′22.0″ W 079°16′16.9″	135	2000–2500	3
Pristine humid tropical forest (Tiputini, 0.76 m)	Amazon basin in the Tiputini River watershed sampling at ground level	S 00°38′12.9″ W 076°08′59.2″	229	2500–3000	4
Pristine humid tropical forest (Tiputini, 10 m)	Amazon basin in the Tiputini River watershed sampled at 10 m	S 00°38′12.6″ W 076°08′59.1″	229	2500–3000	7
Suburban dry montane forest (Tumbaco)	Andean suburb of Quito in a residential area	S 00°13′51.0″ W 078°23′42.8″	2,432	1500–2000	15

Fig. 1. Map of sampling locations. Map of Ecuador indicating the approximate sampling locations.

had intact regrowth forests. Samples were collected during the wet (July) and dry (September and October) seasons at two locations: a natural environment insulated by large areas of intact environment (buffered) versus an area constrained by physical boundaries (edge). The buffered area was surrounded by a minimum of 0.48 km of undeveloped secondary transitional forest in all directions; the edge location was centrally situated in a 50 m-wide tract of secondary forest between a local black-top highway and the Napo River.

The highly altered site is located on the western slope of the Andes Mountains facing the Pacific Ocean in the Itapoá Reserve outside of Puerto Quito at an elevation of 135 m. This area is a highly altered secondary humid tropical forest part of the once massive Chocó Rainforest that ran along the northwestern coast of South America, but is now highly fragmented with large sections having been converted to agriculture, specifically mono-culture plantations of bananas, sugar cane, etc. During sample collection in November of 2009 drought conditions led to crop failures, extremely dusty conditions, and erosion. The sample location was located at the edge of the Itapoá Reserve which is constrained by a dirt road 25 m away and a small agricultural venture.

The final sample site was the suburban deforested environment located at a private residence in Tumbaco, outside of the capital city Quito at 2432 m. This location is marked by a high degree of human development, deforestation, and air pollution intensified by several fires occurring during the time of testing. The location was tested during both the wet (September) and dry (November) seasons.

2.2. Sample collection

TSP samples were collected onto 47 mm Teflon filters (Pall Gellman, Port Washington, NY) fitted on an open face 47 mm filter holder (Advantec, Dublin, CA) using a linear piston pump

(Medo® model VP0435A, Hanover Park, IL). Each sample was collected from 36 m^3 of air accumulated over 24 h with each sampled environment for a minimum of 3 days, generating a minimum of 3 samples per sampling condition. Filter assemblies faced parallel to the ground for both sample collection as well as environmental blank collection. Environmental blanks were collected multiple times at each location and consisted of filter papers exposed to the environment in the filter holder without the application of suction. Unless stated differently, the standardized sampling height was 0.76 m, which for this study was considered ground level. After collection, filter papers were dried at 35 °C for 24 h before being vacuum sealed in plastic petri filter holders for protection from the elements and transport.

2.3. Protein quantification

Bulk protein quantification was performed using the standard protocol (Boreson et al., 2004; Mandalakis et al., 2010; Menetrez et al., 2007) given in the Nanoorange Total Protein Quantitation Kit (Invitrogen®, Carlsbad, CA) and analyzed using a fluorometer (Shimadzu RF 551, Columbia, MD) at the excitation/emission wavelengths of 470/570 nm, respectively. In preparation for the Nanoorange tag the filter papers were extracted once with 7separate 1 mL aliquots of HPLC grade methanol (Fisher Scientific, Pittsburgh, PA) and then ultra-sonicated (Ultrasonic Power Corporation model 2000U 120- V, Freeport, IL) for 30 min. Vials used to prepare the standard protein curve using the protein standard provided in the kit were also prepared with 7 mL of HPLC grade methanol. All of the extracted samples and the standard curve prepared vials were dried at 72 C. Then the standard protocol was followed with the samples being vortexed for 30 s–1 min to ensure the reincorporation of protein into the solution. Reported protein concentrations have been corrected for environmental contamination through the subtraction of the location-specific average environmental blank. The reported concentrations represent the protein concentration collected in a volume of air and may not be completely translatable to ambient air due to sampling bias. However, this bias would be a systematic error true across all of the sample types. This sample analysis approach combines multiple sources of variations from both the analytical error intrinsic to the method as well as natural variations from the environment. The analytical method related error introduced through the use of the Nanoorange Protein Quantitation Kit was $\pm 0.05\ \mu g/m^3$ and the filter blank background was $0.06\ \mu g/m^3$. The Nanoorange method is sensitive to various interferences that may be present in different environments, such as sodium chloride >20 mM and urea >1 M, which must be eliminated or reduced.

2.4. Vegetation indices

Productivity of sampled environments was described using several indices of active vegetation provided by the National Aeronautics and Space Administration on a monthly basis at 1 km^2 spatial resolution (http://neo.sci.gsfc.nasa.gov; Running et al., 2004). These include the normalized difference vegetation index (NDVI), which estimates photosynthetically active vegetation from surface reflectance. Leaf area index (LAI) expresses leaf area per ground area and is derived from spectral imagery and land cover. Net primary productivity (NPP) expresses how much carbon dioxide is absorbed by vegetation and is calculated from NDVI using biome-specific efficiency and respiration conversion factors and is modified by weather observations (Hilker et al., 2012).

3. Results & discussion

3.1. Sensitivity of bioaerosol concentration to environmental variation

This pilot study represents the first appraisal of the natural variations of protein concentrations from TSP in different environments at ground level, looking for trends in their concentrations along with their potential use as environmental indicators. Conducting the study in Ecuador provided two interesting conditions, (1) globally significant richness of environmental and biological diversity within a small, relatively traversable country, and (2) some of the most inhospitable environments for environmental protein preservation. Because protein degradation is exacerbated by both heat and moisture, a tropical rainforest environment poses a worst case scenario for protein preservation.

Several intra-environmental relationships were compared on the basis of aerosol protein concentrations in order to test some of the potential uses of protein concentrations from TSP. The first relationship explored was among ecosystem variation in bioaerosol concentration comparing the pristine, highly altered, regrowth transitional, and suburban deforested sites (Fig. 2). The average concentration of the aerosolized protein was highest in the pristine site ($0.32 \pm 0.09\ \mu g/m^3$) with decreasing amounts being found in the regrowth transitional ($0.17 \pm 0.06\ \mu g/m^3$), the suburban deforested ($0.09 \pm 0.04\ \mu g/m^3$), and the least amount of protein being found in the highly altered forest ($0.07 \pm 0.05\ \mu g/m^3$). Between the two secondary forests, the forest location with a lower degree of human alteration had a higher concentration of bioaerosols than the highly altered forest with a greater extent of human alteration and forest fragmentation. Using one standard deviation the average pristine concentration was distinguishable from all of the other locations. The remaining environment types demonstrate a clear trend in average protein concentrations, although the average protein concentration ranges overlap. The magnitude of the standard deviation was reduced in sample types with larger sample numbers. In comparison to the reported protein concentration of the Brazilian Amazon by Huffman et al. (2012) the environmental protein concentrations at their test location ($0.42 \pm 1.19\ \mu g/m^3$) was slightly higher than the pristine site ($0.32 \pm 0.09\ \mu g/m^3$), although within 1SD (Huffman et al., 2012). The trend in average aerosol protein concentration exhibits

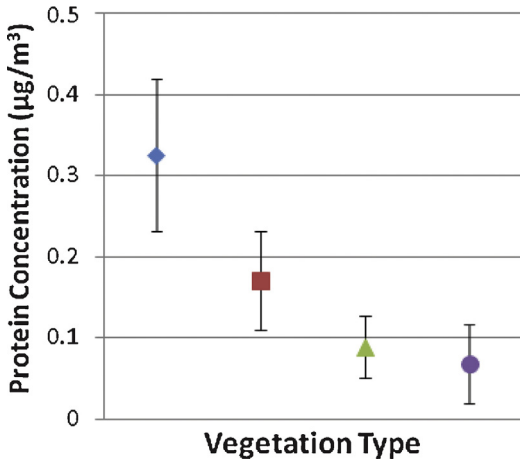

Fig. 2. Comparison of bioaerosol concentrations. Bulk bioaerosol concentrations for four environment types (blue diamond: pristine tropical forest ($n = 4$), red square: secondary transitional very humid forest ($n = 11$), green triangle: suburban dry montane forest ($n = 15$), and purple circle: highly altered secondary humid tropical forest ($n = 3$)) graphed with 1SD. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

sensitivity to a gradient of human alteration as well as gradients in biomass and biodiversity. Future research would focus on exploring and tracking the variations of aerosolized protein concentrations among various ecosystems, while also determining the ratio of protein to TSP collected to gain additional information about the percentage of the local particulate budget represented by aerosolized protein. This is important to determine the effect of TSP concentration on our results.

Next, the influence of seasonal and daily weather on the bioaerosol concentrations was investigated. The dynamics of local TSP concentration, and subsequent environmental protein, depend not only on the amount of protein released into the air via organisms, but also on the complex interaction of TSP with numerous meteorological phenomena, including the impact of mixing (e.g., height of boundary layer, temperature inversions, wind speed and direction) and precipitation (e.g., rain, snow, etc.). Weather phenomena, such as precipitation and wind, are known to have dramatic effects on the presence of aerosolized materials in the air by either using aerosols as nucleation centers and washing them out of the atmosphere or moving them to distal locations (Tong and Lighthart, 2010). A single location was selected for this type of testing, the suburban deforested location (Fig. 3). Comparison among daily observations taken in two seasons (dry season 0.08 ± 0.03 μg/m^3 and wet season 0.10 ± 0.04 μg/m^3) and a variety of weather conditions are inconclusive. The response of ground level bioaerosol concentrations to weather warrants further study. Perhaps, protein concentration variations due to seasonal effects were confounded due to drought conditions during the sample period.

Additionally, the effect of sampling height on the concentration of bioaerosols was explored to evaluate whether aerosol proteins are evenly distributed vertically in the air column. Comparison of samples taken at 0.76 m and 10 m at the pristine site showed that the average concentration of aerosolized protein found at ground level (0.76 m) was higher (0.32 ± 0.09 μg/m^3) than the concentration at 10 m (0.24 ± 0.04 μg/m^3). Possible explanations include sedimentation of larger aerosol material, increased localized plant life at the rainforest floor, forest floor decomposition, and turbulence as well as reduced air flow near to the ground. Because results demonstrated that the distribution of bioaerosols were non-uniform in the vertical direction, future testing is warranted at additional heights and at finer increments throughout the 45 m canopy to better characterize bioaerosol distribution. This result also indicates the need to standardize the sampling height in any monitoring protocol using bioaerosols.

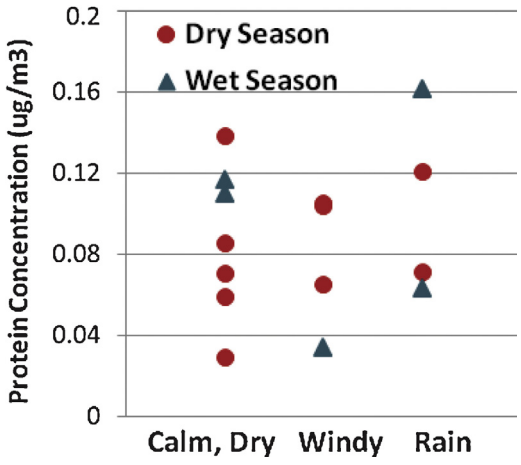

Fig. 3. Effect of seasonal and daily weather. Bioaerosol concentration variations are charted over seasonal and local weather changes at the suburban dry montane forest location. 0.06 μg/m^3 = 1SD for the overall data.

The final intra-environmental comparison examined the impact of edge effects due to fragmentation on bioaerosol material. Physical boundaries (e.g., roads, development activities, rivers and other natural boundaries) are known to cause edge effects such as forest thinning and changes in composition (Ewers and Banks-Leite, 2013). This gradient in forest density and composition is important as different organisms require varying degrees of environmental integrity. Two locations within the regrowth transitional site were used. The first location was buffered by undisturbed forest for at least 0.48 km while the second location was closely constrained by physical boundaries (within 25 m on both sides), e.g., roads and rivers. Samples were collected during both the wet and dry seasons and averaged together for each location. There was a slight decrease in the average quantity of aerosolized protein in the edge location (0.15 ± 0.06 μg/m^3) in comparison to the buffered location (0.19 ± 0.06 μg/m^3), although not statistically distinguishable with 1SD. This could be a result of several causes, including increased air mixing and consequent dilution of bioaerosols as well as lower biomass in the physical boundary itself or an effect of residence time of the particles. While not significant, this result hints at the sensitivity of bioaerosol concentrations to the local environmental setting.

3.2. Relationships of bioaerosol concentration to biological activity

To be an effective monitoring indicator of ecosystem status, bioaerosols must reflect biological activity, a property that is difficult to validate. As a comparison of biological activity among the sampled sites, we accessed several indices of vegetative productivity provided by the NASA Earth Observatory based on MODIS spectral data: NDVI, NPP and LAI, which estimate photosynthetically active vegetation, carbon dioxide absorption, and leaf area per ground area respectively. NDVI is calculated directly from spectral data while NPP and LAI incorporate biome-specific factors describing conversion efficiency, respiration, and vegetation structure as well as ancillary information, such as meteorological data. There are only 14 vegetated biomes defined globally, and only two are represented among the sample sites. Furthermore, these estimates are made for 1-km^2 areas and 1-month intervals. Consequently these vegetation indices represent much lower resolution than the measurements of bioaerosols in space, time and ecosystem distinction. Moreover, they do not include the animal or structural (i.e., non-photosynthetically active) vegetation components of ecosystems, which may contribute to bioaerosol concentrations. Nevertheless, these indices should indicate whether bioaerosol concentrations are consistent with gradients of biological activity.

Data show a similar value of NDVI at the pristine site compared with secondary forests (Fig. 4) despite the expectation that the pristine site would have the highest biological activity. However, this result is consistent with the observation that NDVI becomes saturated in highly vegetated environments and loses the ability to discriminate among them (Justice et al., 2002; Samanta et al., 2012a,b). Consequently, there is a strong relationship between bioaerosol concentrations and NDVI for sites in altered ecosystems ($r^2 = 0.801$) and less so when the pristine site is included ($r^2 = 0.572$). This pattern is repeated for NPP ($r^2 = 0.827$ without pristine site, $r^2 = 0.355$ with pristine site) and LAI ($r^2 = 0.410$ without pristine site, $r^2 = 0.440$ with pristine site). These results suggest that bioaerosols may be more sensitive to changes in pristine forests than are the satellite-based indices, and therefore may be especially useful to detect changes in protected areas.

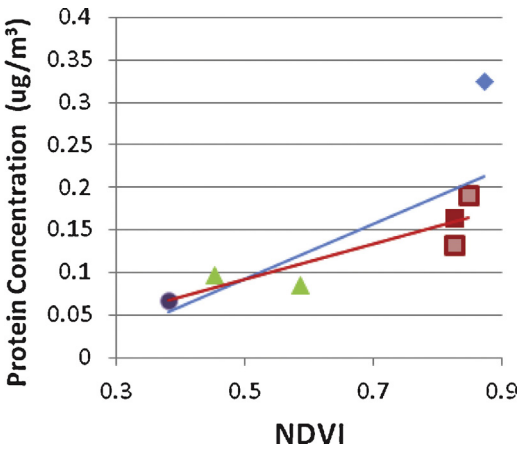

Fig. 4. Graphs of bioaerosol concentrations versus satellite spectral indices. Regression of bioaerosol concentrations on normalized difference vegetation index (NDVI) for all tested locations (blue diamond: pristine tropical forest, solid red square: wet season secondary transitional very humid forest, two-tone red square: dry season secondary transitional very humid forest, green triangle: suburban dry montane forest, and purple circle: highly altered secondary humid tropical forest; $r^2 = 0.572$ for all data points [blue trendline] and $r^2 = 0.801$ for all data points except the pristine humid tropical forest [red trendline]) graphed with 1SD. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. Implications for ecological monitoring

This study represents the first attempt to systematically measure ground level aerosol protein concentrations in several different environments. Measurable concentrations of environmental proteins were detected in all environments tested. Comparisons of the concentration of bioaerosols in the various environments tracked with expected local primary production levels. Intra-environmental comparisons of sampling height, and edge effects suggested sensitivity of bioaerosols to observed local environmental differences at higher spatial resolution than readily available remotely sensed indicators. When compared to current satellite based spectral techniques such as NDVI, NPP, and LAI bulk bioaerosol concentrations correlate well with the exception of highly vegetated environments such as the pristine humid tropical forest where bioaerosols show greater sensitivity to biomass. The ability of the technique to track environment specific trends that are consistent with satellite indices through repeat measurements at each site demonstrate a level of robustness as well as a limited impact of events like weather and the phenology of flowering.

Biodiversity is commonly described as a measure of ecosystem health, yet it is extremely difficult to quantify, especially by cost-effective means as required for ecological monitoring (Running et al., 2004). Land use and climate change, erosion, nitrogen deposition, biotic exchange, and increases in atmospheric CO_2 have been identified as the primary drivers of change in global biodiversity while NPP is thought to integrate these drivers (Bradley et al., 2011; Running et al., 2004; Sala et al., 2000). This study shows that bioaerosol concentration is relevant to describing biodiversity and ecosystem status because it is sensitive to at least two of these drivers, land use and climate, and is strongly related to NPP. Bioaerosols have the added advantages of being relatively inexpensive to measure and having higher sensitivity to changes in pristine forests than satellite-based indices.

4. Conclusions

This limited pilot study has demonstrated that simple, relatively inexpensive bulk bioaerosol monitoring can contribute to the understanding of local environmental changes by increasing the local resolution (e.g., edge effects and fragmentation), phenological tracking of plants, and the z-axis structure of forests, especially dense canopy forests. Further elucidation of the role of bioaerosols in the environment is necessary to quantify their sensitivity and resolution. Also, the collection of a greater number of samples per location would be helpful to reduce the standard deviations in each sample type as well as to develop a finer resolution to track changes, like proximity to physical boundaries and height. To allow for comparison, these studies can be performed in concert with other ground level methods such as intensive plot-based vegetation description and allometry. Capitalizing on the low cost associated with equipment and analysis (under $3,500 for the entire study) as well as the simplicity of sampling; this technology is a natural and logical tool to foster partnerships with local communities to collect samples that are sent to a centralized laboratory for analysis. Bulk bioaerosol monitoring could provide a ground level integrative indicator of ecosystem status, which could be used to help paint a more holistic view of the state of the environment. Future applications using bioaerosols material include tracking local environmental recovery, local human health issues, and the impact of human activities.

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