Wading bird guano contributes to Hg accumulation in tree island soils in the Florida Everglades

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A B S T R A C T

Tree islands are habitat for wading birds and a characteristic landscape feature in the Everglades. A total of 93 surface soil and 3 soil core samples were collected from 7 degraded/ghost and 34 live tree islands. The mean Hg concentration in surface soils of ghost tree islands was low and similar to marsh soil. For live tree islands, Hg concentrations in the surface head region were considerably greater than those in mid and tail region, and marsh soils. Hg concentrations in bird guano (286 µg kg⁻¹) were significantly higher than those in mammal droppings (105 µg kg⁻¹) and plant leaves (53 µg kg⁻¹). In addition, Hg concentrations and δ⁵³⁴N values displayed positive correlation in soils influenced by guano. During 1998—2010, estimated annual Hg deposition by guano was 148 µg m⁻² yr⁻¹ and ~8 times the atmospheric deposition.

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1. Introduction

The Florida Everglades is the largest freshwater wetland in the continental United States. High Hg concentrations in fish and wildlife have been of particular concern (Axelrad et al., 2006; Rumbold et al., 2007). The Hg concentrations in the majority of largemouth bass (Micropterus salmoides) from the Everglades protection area have exceeded the USEPA methylmercury (MeHg) criterion of 0.3 mg kg⁻¹ in fish tissue since 1998 (Gabriel et al., 2010). The Hg in the Everglades originates from local, regional and global sources (Axelrad et al., 2006). Atmospheric deposition, primarily inorganic Hg, contributes over 95% of Hg input to the Everglades (Axelrad et al., 2006; Liu et al., 2008a). Deposition rates for atmospheric Hg vary between 15 and 25 µg m⁻² yr⁻¹ in southern Florida (Rumbold et al., 2007). Soil is the largest sink for Hg, with ~80% of seasonally deposited Hg being entrapped in soils (Liu et al., 2009, 2010).

Tree islands are a characteristic landscape feature in the Everglades. The total area of tree islands varies from 14% in the northern Everglades to 3.8% in the central Everglades (Brandt et al., 2000; Wetzel, 2003; Wetzel et al., 2011). Tree islands in the central and southern Everglades typically consists of head, middle and tail regions (Mason and Valk, 2002). The head is the most elevated part of the island, containing the largest trees in terms of both height and trunk diameter. The middle and tail region of tree island usually are vegetated by low stature trees and a dense herbaceous understory including shrubs, forbs ferns, shrubs and sawgrass (Mason and Valk, 2002; Sah, 2004). In addition, most of wading bird nests are located in the head region of tree islands due to their favorable vegetation communities (Orem et al., 2003).

The Everglades includes four management units: Water Conservation Areas (WCA-1, WCA-2 and WCA-3) and the Everglades National Park (ENP). Tree islands in WCA-2A and 3A have suffered the most change and lost their elevation and woody vegetation due to persistent and deep flooding, and they are known as “ghost” islands (Hofmockel et al., 2008). Ghost tree islands have disappeared from the landscape and can be located only as scars in aerial photographs. The live tree islands are not degraded and have healthy vegetation community. Most of the live tree islands are located in WCA-1, 3A, 3B and ENP. Live tree islands have more woody vegetation and wading bird nests than ghost tree islands, especially in their head regions (Sklar et al., 2011).
Tree islands are an important habitat for wildlife, especially large populations of wading birds (Gawlik and Rocque, 1998). WCA-1, WCA-3 and ENP are the primary nesting regions for wading birds in the Everglades. In 2010, ENP supported the most nests (37%) followed by WCA-1 (34%) while WCA-3 supported the lowest number of nests (29%) (Cook and Kobza, 2010). Wading bird colonies located in central and south portion of the Everglades (WCA-3B and ENP) were at higher risk to Hg exposure than those colonies feeding elsewhere in the Everglades due to high Hg concentration in fish (Stober et al., 2001).

Recent research indicates that tree islands are biogeochemical hotspots that sequester nutrient within the Everglades (Wetzel et al., 2005, 2011). The concentration distribution of total Hg and MeHg across Everglades marsh soils has been documented (Arfwidson et al., 2000; Cohen et al., 2009; Liu et al., 2009), but the spatial distribution of Hg in tree islands soils is unknown. It is known that seabirds transport contaminants to their nesting sites via deposition of guano, carcasses, and shed feathers (Blais et al., 2005; Brimble et al., 2009; Evenset et al., 2007; Foster et al., 2011). A positive correlation between Hg concentrations and seabird populations was observed in Arctic ponds in Devon Island, with ponds most affected by seabird guano containing 25 times higher Hg concentrations than unaffected sediments (Blais et al., 2005). Deposition of wading bird guano has been hypothesized for nutrient accumulation in tree island soils in the Everglades, particularly phosphorus (Wetzel et al., 2011, 2005). Besides nutrients, wading birds in the Everglades may transport Hg to their nesting site via guano. This is because wading birds in the Everglades prey on invertebrates and fish from the marsh and accumulate high Hg concentrations in their feathers and eggs (Herring et al., 2009). However, Hg deposition from wading bird guano in the Everglades tree island soils has not been reported.

Stable nitrogen isotope analysis is a powerful tool to document the trophic position of animals, as consumer δ15N values display an average increase of 3.4‰ per trophic level along food chains (Post, 2002). Elevated concentrations of organic and metal pollutants associated with high δ15N in soil and sediment affected by seabirds have been reported (Blais et al., 2005; Brimble et al., 2009; Foster et al., 2011). The correlation of δ15N and Hg was used to identify trophic linkages and Hg transfer in organisms living near arctic seabird colonies (Choy et al., 2010). In addition, Hg concentrations and δ15N values are significantly positively correlated in seabird-affected ponds (Blais et al., 2005; Evenset et al., 2007).

The objective of this study was to assess the spatial distribution of Hg in tree island soils and the influence of wading bird guano deposition on Hg accumulation in tree island soils. This was accomplished by 1) comparing Hg concentrations in tree island soils with and without wading bird guano, 2) investigating the relationship between Hg concentration and δ15N values in tree island soils affected by wading bird guano, and 3) calculating the wading birds guano deposition rates to tree island soils during 1998–2010.

2. Materials and methods

2.1. Study site

Forty-one tree islands representing a broad range of sizes and hydrologic regimes in the Everglades were selected for this study. They were from 3 WCAs in the
tions with wading bird guano. The plant leaves collected from tree islands including excrement from other animals were also collected to compare their Hg concentrations. Mammal droppings samples were from white-tail deer (*Odocoileus virginianus*).

2.3. Hg deposition via wading bird guano

On dropping appearance and previous reports on wildlife surveys for tree islands in WCA-3A and 3B, the species included great egrets (*Ardea alba*), white ibis (*Eudocimus albus*), snowys egrets (*Egretta thula*), little blue herons (*Egretta caerulea*), and tri-colored herons (*Egretta tricolor*). The bone samples of wading birds consisted of great egrets (*Ardea alba*), and white ibis (*Eudocimus albus*). Plant leaves and excrement from other animals were also collected to compare their Hg concentrations with wading bird guano. The plant leaves collected from tree islands including wax myrtle (*Morella cerifera*), coo plum (*Chrysobalanus icaco*), willow (*Salix caroliniana*), button bush (*Cephalanthus occidentalis*) and hard fen (*Blechnum sp.)*. Mammal droppings samples were from white-tail deer (*Odocoileus virginianus*), eastern cottontall rabbit (*Sylvilagus floridanus*), feral pig (*Sus scrofa*), black bear (*Ursus americanus*) and raccoons (*Procyon lotor*). Mammal identification was based on dropping appearance and previous reports on wildlife surveys for tree islands in the Everglades.

2.2. Sample collection

Soil samples were taken from 7 ghost and 34 live tree islands between 2009 and 2010. Transects were set up at the head, middle and tail of ghost and live tree islands from WCA-2, 3A and 3B. A total of 93 surface soil samples (top 10 cm) were collected. Not all tree islands had mud and tail sections, with several small tree islands having only a head section (WCA-1 and ENP). In addition to surface soils, three soils cores (50 cm long) were obtained from the head, mid and tail section of a live tree island in WCA-3B. The head region of the tree island had wading bird nests. Soil core samples were sectioned at 2 cm intervals for Hg analysis.

Bird guano samples were from mixed species of wading birds from several tree islands in WCA-3A and 3B, the species included great egrets (*Ardea alba*), white ibis (*Eudocimus albus*), snowys egrets (*Egretta thula*), little blue herons (*Egretta caerulea*), and tri-colored herons (*Egretta tricolor*). The bone samples of wading birds consisted of great egrets (*Ardea alba*), and white ibis (*Eudocimus albus*). Plant leaves and excrement from other animals were also collected to compare their Hg concentrations with wading bird guano. The plant leaves collected from tree islands including wax myrtle (*Morella cerifera*), coo plum (*Chrysobalanus icaco*), willow (*Salix caroliniana*), button bush (*Cephalanthus occidentalis*) and hard fen (*Blechnum sp.*) Mammal droppings samples were from white-tail deer (*Odocoileus virginianus*), eastern cottontall rabbit (*Sylvilagus floridanus*), feral pig (*Sus scrofa*), black bear (*Ursus americanus*) and raccoons (*Procyon lotor*). Mammal identification was based on dropping appearance and previous reports on wildlife surveys for tree islands in the Everglades.

2.4. Hg analysis and quality assurance

Total Hg concentrations in soils, plant leaves, mammal dropping, wading bird guano and bones were digested with 7:3 (v/v) concentrated HNO₃/H₂SO₄ acids at 110 °C overnight according to EPA Method 3051 and analyzed using hydride generation atomic fluorescence spectrometry (HGAFS). Soil plant leaves, mammal droppings, wading bird guano and bones were oven-dried (60 °C) and ground to fine powder (<2 mm) before digestion. Hg concentrations in all samples were calculated based on dry weight. Strict quality control procedures were followed during the sample analysis. Quality control samples, including two method blanks, matrix spikes, lab duplicates, and certified reference material SMR 2709, were analyzed every 30 samples. In all method blanks, total Hg concentrations were below detection limits of HGAFS (0.01 µg L⁻¹ for water and 2 µg kg⁻¹ for soil). Mean coefficients of variation for duplicate samples were low (6.70–13.2%) and recoveries for matrix spikes or certified reference material were within the acceptable ranges (80–120%). Instrument performance was checked by running an intermediate calibration standard for every 15 samples. All calibration standard checks were within the acceptable range (90–110%).

2.6. Statistical analysis

The Hg concentration patterns in surface soils of tree islands were examined by a probabilistic model, which assumed the log transformed data are all uncorrelated. Comparison of Hg concentrations in different regions of ghost and live tree islands soils was performed by the student t-test, and comparison of Hg concentrations and δ¹⁵N values in head, mid and tail section in soil core samples was performed by the paired t-test. Regression analyses between Hg concentrations and soil property (TP, TN, SOM, and BD) were also conducted. All correlation analyses were conducted on log-transformed data. The normality test (Shapiro–Wilk statistic) and homogeneity variance test (Levene’s test) indicated all variables met the assumption of normality and homogeneity after log transformation. Comparisons of Hg concentrations in plants, mammal dropping, wading bird guano and bones in tree islands were performed by the student’s t-test. A p value less than 0.05 was considered significant. All statistical analyses were carried out with SAS 9.3 or Sig-maPlot 11.0.

### 3. Results and discussion

#### 3.1. Spatial Hg distributions in tree islands soils were different from marsh soils

Surface soil samples (*n = 93*) were collected from 41 tree islands across the Everglades. The soil Hg concentrations ranged from 79 to 842 µg kg⁻¹, averaging 209 µg kg⁻¹ (*Table 1*). The mean soil Hg concentration was 155 µg kg⁻¹, with a standard deviation of 280 µg kg⁻¹.
concentrations from tree islands in WCA-1 (557 ± 58 μg kg⁻¹), WCA-3B (441 ± 65 μg kg⁻¹) and ENP (356 ± 21 μg kg⁻¹) were significantly higher than those in WCA-2A (142 ± 12 μg kg⁻¹) and WCA-3A (165 ± 24 μg kg⁻¹) (Fig. 2). A total of 9 Hg hotspots (defined as Hg ≥ 400 μg kg⁻¹) were identified in the head regions, of which 5 were in WCA-3B, 3 were in ENP, and 1 in WCA-1. The highest soil Hg concentration (840 μg kg⁻¹) was observed in WCA-3B whereas Hg concentrations in 7 ghost tree islands soils from WCA-2A were all < 400 μg kg⁻¹ (Fig. 1).

Hg concentrations in the marsh soils were generally higher in the north and central Everglades (WCA-1 and 2, 3) and lower in the southern regions (ENP) (Cohen et al., 2009; Liu et al., 2009; Stober et al., 2001). Higher atmospheric deposition of Hg in the northern Everglades leads to higher Hg levels in the north Everglades marsh soil (Arfstrom et al., 2000; Cohen et al., 2009). If atmospheric deposition were the only source of Hg to the Everglades, then soils in tree islands and marshes should have received similar amounts of Hg, and Hg spatial distribution in different hydrological regions (WCAs and ENP) of the tree island and marsh soil should be similar. Compared to marsh soils, the Hg concentrations in tree island soils followed a different pattern with high values in the northern and southern Everglades (WCA-1, WCA-3B and ENP). These observations indicated that in addition to atmospheric deposition, the high Hg concentrations in tree island soils may have derived from other sources. In addition, normal probability plots for surface soil Hg did not fit a straight line (data not shown), suggesting more than one source of Hg in these samples. Deposition of wading bird guano and other animals’ droppings enriched with Hg is a possible source of Hg accumulation in tree island soils.

3.2. Hg concentrations in surface soils from ghost tree islands were low

The Hg concentrations from the 7 ghost tree island soils (n = 21) from WCA-2A ranged from 89 to 265 μg kg⁻¹, averaging 142 μg kg⁻¹, which was similar to marsh soils (146 μg kg⁻¹) (Cohen et al., 2009; Liu et al., 2008b). Cohen et al. observed Hg values of 2–917 μg kg⁻¹, averaging 162 μg kg⁻¹ (Cohen et al., 2009). Liu et al. (2008b) reported Hg concentrations in the Everglades marsh soils range from 9.3 to 350 μg kg⁻¹, averaging 130 μg kg⁻¹. In addition, the average bulk density of the ghost tree island soils (0.10 g cm⁻³) was similar to the surrounding slough (0.08 g cm⁻³), and was significantly lower than those in live tree islands in WCA-3A (0.3–0.6 g cm⁻³) (Sklar et al., 2011). The mean soil Hg concentrations in the head, mid and tail regions were 122, 149 and 157 μg kg⁻¹. There was no significant difference in soil Hg concentrations among head, mid and tail regions (p < 0.05) (Fig. 3a). The head regions of ghost tree islands in WCA-2A mostly had no woody vegetation, with the most common species being sawgrass and herbaceous species (Sklar et al., 2011). The absence of woody vegetation and dominance of sawgrass in the head region of ghost tree islands led to the absence of wading birds colonies and guano deposition. These observations indicated that the absence of wading bird guano could contribute to the low Hg concentrations in surface soils of ghost tree islands. Other process such as flooding and fire could also lead to low Hg concentration in the degraded tree islands, which need further study.

3.3. Soils from head regions of live tree islands were enriched with both Hg and δ¹⁵N

The Hg concentrations in 34 live tree island soils (n = 72) from WCA-1, 3A, 3B and ENP ranged from 79 to 842 μg kg⁻¹, averaging 234 μg kg⁻¹. The mean Hg concentrations in the head region (317 μg kg⁻¹) from live tree islands were significantly higher than those in mid (155 μg kg⁻¹, p < 0.01) and tail (132 μg kg⁻¹, p < 0.01) regions (Fig. 3b). In addition, the Hg concentration (317 μg kg⁻¹) in the head region of live tree islands was significantly higher than that of ghost tree islands (122 μg kg⁻¹). Besides surface soils from 34 live tree islands, we also compared Hg concentrations in three soil core samples (50 cm) from the head, mid and tail regions of a live tree island in WCA-3B. The head region of this live tree island is particularly conducive for wading bird reproduction and large numbers of nests are concentrated in this region. The mean Hg concentrations in the head region in the surface 10 cm were 589 μg kg⁻¹. In addition, the mean Hg concentrations in the head region soil core (389 μg kg⁻¹) were significantly higher than that in mid (124 μg kg⁻¹, p < 0.01) and tail region (106 μg kg⁻¹, p < 0.01) (Fig. 4a). In addition to high Hg, the δ¹⁵N values in soils from the head regions of live tree islands were also high. Marsh soil and plants are depleted in δ¹⁵N, with a range from −1 to 2‰ (Sklar et al., 2011). The mean δ¹⁵N value of head surface soils from live tree islands was 7.5‰ (Table 1). The δ¹⁵N values in the head region (9.7‰) were significantly higher than that in mid (5.5‰, p < 0.01) and tail region (4.9‰, p < 0.01) (Fig. 4b). The δ¹⁵N of guano samples collected close to wading bird colonies in the Everglades ranged from 7.8 to 10.6‰, averaging 8.8‰ (Table 1). The high δ¹⁵N values of live tree island soils may result from guano deposition from wading birds. Other sources cannot lead to the observed δ¹⁵N enrichment...
in the soils due to their low trophic positions (e.g., tree leaves and mammal droppings) or low population size of higher trophic level predators (e.g., bears) (Axelrad et al., 2013). Hence, δ^{15}N was used as a tracer for wading bird guano in this study. The high δ^{15}N values in the head regions of live tree islands indicated the presence of wading bird guano in those soils.

In our study, δ^{15}N was positively correlated with soil Hg concentrations in surface samples of live tree island (R^2 = 0.52, p < 0.01) and the core soil samples (R^2 = 0.27, p < 0.01) from a live tree island with wading birds colony (Table 2; Fig. 5 a, b). Soil Hg concentrations and δ^{15}N values were not significantly correlated in the mid and tail regions (R^2 = 0.01–0.18, p > 0.01), which were not influenced by wading bird guano (Fig. 5c, d). These results indicated that high Hg concentrations in the soil may have been influenced by bird guano. To support this hypothesis, we analyzed the Hg concentrations in surface soils (n = 5) from a live tree island in WCA-1, which is known to be nested by great egrets. The mean Hg concentrations in those soils were high, averaging 454 μg kg^{-1}, which supported our hypothesis.

### 3.4. Hg deposition via wading bird guano

Wading birds can accumulate Hg in their feathers, bones, and guano. The guano and bone samples in this study represented mixed wading bird species, including great egrets, snowy egrets, white ibis, little blue herons and tri-colored herons. These species feed on invertebrates and fish in the Everglades and are positioned near the top of the aquatic food chain. Hg biomagnifies as it progressively moves up the food chains of the Everglades. The Hg concentration in wading bird guano, bones and mammal dropping were 286, 89 and 105 μg kg^{-1}, respectively (Table 1). The Hg concentrations in wading bird guano were significantly higher than those in wading bird bones and mammal droppings (Fig. 6, p < 0.05).

The Hg concentrations in tree island plants (wax myrtle, cocom plum, willow, button bush and hard fen) were low, ranging from 20 to 128 μg kg^{-1}, averaging 53 μg kg^{-1}. The Hg concentrations in plant leaves were higher than those in cattail and sawgrass leaves (1.0–13 μg kg^{-1}) (Stober et al., 2001). Assuming Hg concentration of 53 μg kg^{-1} in the leaves, and dry mass in aboveground litterfall of 500 g m^{-2} yr^{-1} (Guentzel et al., 1998), the calculated annual Hg deposition was 27 μg m^{-2} yr^{-1}. This is comparable to the wet Hg deposition in southern Florida (19 μg m^{-2} yr^{-1} in 2006) (NADP, 2007). Hence, Hg deposition through plant leaves in tree islands in the Everglades may not be a significant source.

Considering the high Hg concentrations in wading bird guano and the large population of wading birds in tree islands, we calculated the guano Hg deposition in a tree island with a wading bird colony. Based on measured average Hg concentration in guano, guano excretion rates and wading bird nest numbers of the Hidden colony (data not shown), wading bird guano Hg deposition rate was as high as 790 μg m^{-2} yr^{-1} in 2009 (more than 42 times the average atmospheric deposition rate). The average guano Hg deposition for 1998—2010 was 148 μg m^{-2} yr^{-1} (~8 times that average atmospheric deposition rate). The estimated wading guano Hg deposition in tree islands suggests that wading birds play an important role in Hg redistribution within the Everglades ecosystem, and Hg deposition from wading bird guano at densely populated colonies can be high.

Our calculation provided a conservative estimate about guano Hg deposition to the tree island soils in the Everglades. The historical Hg deposition by guano could be much higher than 148 μg m^{-2} yr^{-1}. This is because the Hg concentrations in the Everglades fish and wading birds have undergone a significant decline since 1990s. Hg concentrations in fish from WCAs have declined by ~40–80% over the past decade (Axelrad et al., 2008). Thus Hg concentrations in wading bird guano before 2010 could be much higher than 286 μg kg^{-1}. In addition, the Hg deposition rate by wading bird guano at tree islands could change among different years and locations due to the variability of guano Hg concentrations and nest numbers. Unlike inorganic Hg in atmosphere deposition, MeHg concentration in wading bird guano could be high. This was because MeHg is the major form of Hg in fish, and bird may accumulate MeHg through food chain. For example, the seabird guano from Xisha Island, China contains 45% MeHg, which

![Fig. 4. Hg concentrations (a) and δ^{15}N values (b) in soil cores from a live tree island in WCA-3B.](image-url)
is identified as the major source of MeHg in ornithogenic coral sediments (Chen et al., 2012). The dominant form of Hg in the Everglades marsh soil is inorganic Hg (\( \sim 99\% \)), mean MeHg concentration was very low (1.4 \( \mu \)g kg\(^{-1}\)) (Liu et al., 2008b). Hence wading bird guano could elevate MeHg concentration in tree island soils.

### 4. Environmental implications

Our results provide novel information on Hg concentrations in soils, plants, mammal droppings and wading bird guano in tree islands in the Everglades. Guano deposition by wading birds played an important role in transporting Hg to tree island soils. These findings have important implications for Hg management in the Everglades. The head regions of live tree islands are important habitats for animals (alligators, deer, bear, and panther), especially during the wet season. Although wading bird guano deposition redistributed only a small fraction of the Hg in the Everglades, Hg deposition at sites with large numbers of nests could be high. Hg concentrations and deposition rates of guano may differ between regions, years and species. The contribution of wading bird guano to the relative Hg budget in the Everglades could be applied to other wetland ecosystems. Future research should evaluate the level of highly toxic MeHg in tree island soils, and Hg bioavailability in wading bird guano and tree island soils, and assess the potential ecological health risks from Hg contamination in tree island soils.

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