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Tracing the Uptake, Transport, and Fate of Mercury in Sawgrass (*Cladium jamaicense*) in the Florida Everglades Using a Multi-isotope Technique

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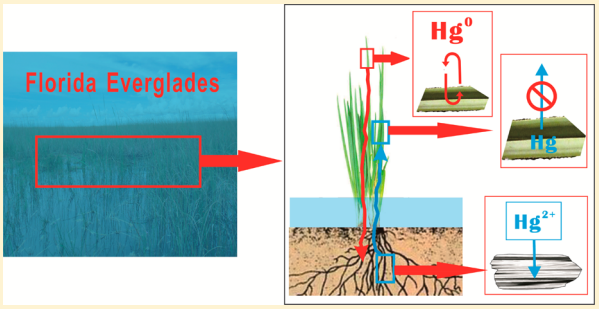
Supporting Information

ABSTRACT: The role of macrophytes in the biogeochemical cycle of mercury (Hg) in the Florida Everglades is poorly understood. Stable isotope tracer techniques were employed to investigate Hg uptake by sawgrass (*Cladium jamaicense*) from soil and atmospheric pathways and the fate of Hg after absorption. Our results suggest that soil spiked $^{201}Hg^{2+}$ was rapidly taken up by roots and transported to aboveground parts. The spiked ^{201}Hg that was transported to the aboveground parts was trapped; no release of the spiked ^{201}Hg from the leaf to the air was detected. Atmospheric $^{199}Hg^0$ exposure experiments revealed that the majority of the previously deposited $^{199}Hg^0$ taken into the leaf was fixed, with a very limited proportion (1.6%) available for re-emission to the atmosphere. The percentage of $^{199}Hg^0$ fixed in the leaf will help reduce the model uncertainty in estimating the Hg^0 exchange over the air−vegetation surface. We propose that sawgrass needs to be viewed as an important sink for atmospheric Hg^0 in the regional Hg mass balance; this would have important implications for the critical loads of Hg to the Everglades. The multi-isotope tracer technique could be an effective tool to identify the role of plants in biogeochemical cycling of Hg in other ecosystems.



1. INTRODUCTION

Mercury (Hg) has received great attention as a global pollutant due to its ability to undergo methylation, accumulation, and biomagnification in aquatic food chains.[1] Wetlands are widely known as sites for methylmercury (MeHg) production[2−4] and are generally considered net sources of MeHg.[5] In the Florida Everglades, MeHg is biomagnified and can reach high levels in fish and fish consumers.[6,7] The death of a Florida panther, a federally endangered species, had been attributed to Hg poisoning.[8,9] Emergent macrophytes in the Everglades are important contributors to Hg exchange between the atmosphere and substrate sediments.[4,10−13] Previous studies showed that Hg emission from water and, in particular, transpiration through macrophytes, represents an important pathway of Hg removal, with 10% of seasonally deposited Hg being evaded out of this ecosystem.[4,12−14] The exact source of the Hg^0 emitted from macrophytes is unknown, but origins either from soil, water, or from atmospheric deposition (previously deposited Hg^0) are reasonable. Numerous studies have suggested that vegetation could assimilate both inorganic and organic Hg from soil solution through their roots and transport the Hg to the foliage; this Hg may then be emitted to the atmosphere via the stomata.[15,16] While the emission of Hg^0 from the foliage is undisputed, previous studies have not been able to distinguish the source of re-emitted Hg due to technical limitations.

Using a stable isotope tracer technique, Mao et al.[17] provided an understanding of uptake pathways of Hg within sawgrass in the Everglades. However, it is still unknown whether the Hg^0 emitted from foliage was derived from the atmospherically deposited Hg or from Hg taken up from the soil solution and subsequently converted to a volatile form. Based on the measurements of Hg accumulation in foliar over a growing season, Rea et al.[18] suggested that only 25% of Hg^0 (f_{fixed})

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through atmospheric dry deposition to canopy is permanently fixed into leaf tissues and not available for re-emission.[18] Recently, an updated model was developed to estimate the Hg^0 exchange over the air−stomata surface.[19,20] It is clear that the estimated Hg^0 flux over the air−stomata surface is dependent on the f_{fixed} during the calculations. Unfortunately, direct evidence for the fraction of Hg^0 fixed into tissues is lacking. Therefore, large uncertainty was unavoidable when estimating the global natural emission of Hg^0 over the air−foliar surface.[19−21]

The role of macrophytes within the Everglades as sources or sinks of atmospheric Hg is not well understood, and several key questions have not been answered. Do plants simply recycle Hg by taking it up from the soil and then return it by way of litterfall, or do leaves directly capture atmospheric Hg and re-emit it to the air as a new source? To address these questions, this study focused on the role of sawgrass in Hg cycling in the Everglades, as sawgrass is the dominant macrophyte in the Everglades wetland, covering approximately 50−75% of the landscape.[22,23] In order to characterize the transport of Hg across air−vegetation−soil interfaces, multi-isotope tracer techniques were used to trace the different processes. We quantified the uptake and transportation of Hg within tissues of sawgrass in a laboratory controlled system using an enriched elemental Hg vapor ($^{199}Hg^0$) and a mercuric Hg isotope ($^{201}Hg^{2+}$) as isotopic tracers. The Hg flux between the foliage and atmosphere was measured using a dynamic flux bag. The objectives of this study were to (1) assess the uptake and transportation of Hg species in sawgrass tissues via root and atmospheric pathways; (2) quantify the storage of Hg in tissues of sawgrass; and (3) elucidate whether sawgrass is a net source or sink of Hg in the Everglades ecosystem.

2. MATERIALS AND METHODS

2.1. Chemicals and Materials. Enriched ^{199}HgO and ^{201}HgO was purchased from Oak Ridge National Laboratory (Oak Ridge, Tennessee). $^{199}HgCl_2$ and $^{201}HgCl_2$ solution were prepared by dissolving ^{199}HgO and ^{201}HgO in 10% HCl (*v*/*v*), respectively. The enriched $^{199}Hg^0$ was generated from a Hg vapor generator during the course of atmospheric exposure experiments. Other reagents used in this study were at least of analytical grade and were purchased from Fisher Scientific Company L.L.C. USA.

2.2. Soil and Sawgrass Plants. Wetland peat soil samples were collected from an Everglades marsh (25°45′57″ N, 80°29′54.2″ W). The soil was air-dried and sieved (4 mm mesh) for homogeneity. Sawgrass (*Cladium jamaicense*) seedlings with 4−5 mature leaves (20−40 cm in length) were purchased from Aquatic Plants of Florida, Inc., Sarasota, FL, USA. Approximately 290 g (dry weight) homogenized soil was transferred into polyethylene pots (15 cm deep × 15 cm diameter). Three sawgrass seedlings were planted into each pot. In order to simulate natural growing conditions, the sawgrass pots were kept outdoors on shelves suspended in a water-filled polyethylene tank (3410 L, 1 m deep × 2.1 m diameter) in a fenced yard located at Florida International University, Miami, Florida. Flooded soil conditions, with ∼2 cm of water above soil surface in sawgrass pots, were maintained during the experiment. Tap water was used to replenish water in the tanks and in the experimental pots throughout the experiment. The plants were allowed to stabilize in the tanks for 1 month prior to experimentation.

2.3. Soil $^{201}Hg^{2+}$ Spiking Experiments. In order to investigate the uptake and transport of Hg across soil−vegetation interface and subsequent fates of Hg after absorption, two experimental groups of 9 pots each (9 pots for control and 9 pots for treatment groups) were carefully designed in this study. In detail, enriched isotope of $^{201}Hg^{2+}$ tracer solution prepared in deionized water ($^{201}Hg^{2+}$ = 16 mg L^{-1}as Hg) was spiked into the soil in the pots. Spiking solution of 20 mL was applied using a disposable syringe at five positions evenly distributed in each pot in soil depth of 5 cm. Previous study confirmed that the majority (>90%) of total Hg (THg) in sawgrass leaves was obtained from atmospheric Hg, rather than from soil source.[17] To obtain sufficient isotopic signal, the concentration of spiked $^{201}Hg^{2+}$ was designed to be 1100 μg kg^{-1} for Hg for each of the 9 treatment pots, which was approximately 4 times higher than THg levels in Everglades soil.[4] Immediately after spiking, the surface of the pots was covered with plastic lids to reduce the possible loss of Hg through emission from soil, consequently changing Hg isotope ratios in above ground part of plant. The lids contained a center hole through which the leaves emerged. The center hole was plugged using a polyethylene foam collar around the leaves.

Sampling was initiated the first day after spiking with the Hg isotopes (day 1) and, thereafter, at 30 and 60 days. Hg vapor flux over sawgrass leaves (1 pot) was quantified for 24 h at each sampling time as described in the Supporting Information (SI). After the gas-exchange experiments were complete, the sawgrass plants and all the soil from 3 pots per treatment were collected for THg isotopic analysis. Detailed information on sample collection and preparation is given in the SI.

2.4. Atmospheric Hg Isotope ($^{199}Hg^0$) Exposure Experiments. As shown in SI Figure S1, a flux bag coupled with a stable Hg isotope addition technique was used to directly track the short-term uptake and re-emission of Hg^0 at the air−leaf surface. Plant leaves were exposed to isotopically enriched gaseous elemental $^{199}Hg^0$(g) for 24 h. Exposure experiments were conducted under natural plant growth conditions and atmospherically relevant Hg^0(g) concentrations in a custom-made flux bag (SI Figure S1). The enriched $^{199}Hg^0$ stable isotope was pumped into the flux bag from a Hg vapor generator (Gas Liquid Separator, M055G003, P S Analytical, UK). The generated $^{199}Hg^0$ was evaded by N_2 gas (20 mL min^{-1}) and mixed with natural air delivered from a pump, then transferred to a chamber from its top through two evenly distributed outlet Teflon pipes at a flow rate of ∼11 L min^{-1} using a vacuum pump (P-7902-00, Cole Parmer Instrument Corporation, USA). The target enriched $^{199}Hg^0$ concentration in the flux bag was jointly controlled by the flow rate of the carrier gas (ambient air) and the mass of the generated $^{199}Hg^0$.

We quantified the mass of enriched $^{199}Hg^0$ taken up by sawgrass leaves during 24 h exposure (SI Figure S2). After exposure for 24 h, enriched $^{199}Hg^0$ flux over the leaf−air surface was then measured for 24 h using the flux bag to determine the release of ^{199}Hg to the atmosphere in the subsequent day (day = 1). Thereafter, the re-emission of the enriched ^{199}Hg was sampled again at days 30 (30 days after the exposure experiment) and 60. A detailed description of experimental design is described in the SI, including quantifying the mass of enriched $^{199}Hg^0$ taken up by sawgrass and the re-emission of the ^{199}Hg from leaf to the air. To determine the location and transport of enriched ^{199}Hg within sawgrass plant, sawgrass and corresponding soil (3 triplicate pots) were collected following

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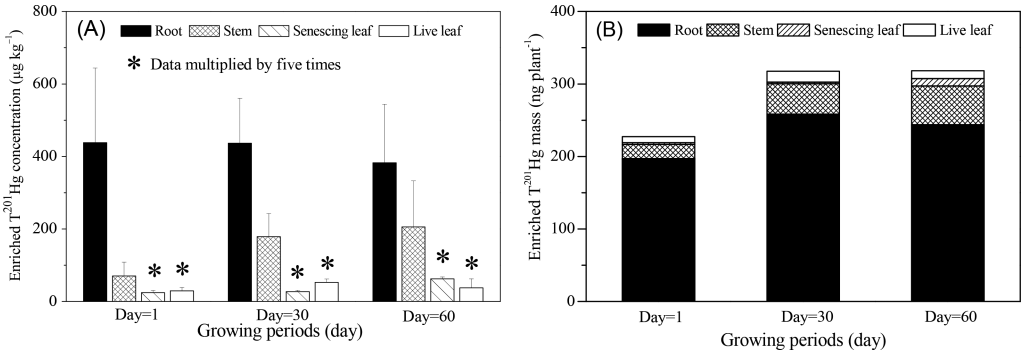

Figure 1. Concentrations (A) and total mass (B) of enriched $T^{201}Hg$ in sawgrass tissues (n = 3) at different sampling times.

each of the flux measurements for enriched THg isotopes analysis, as described in detail in the SI.

After each of the flux measurements, the flux bag was carefully cleaned and stored in a Hg free room (see details in the SI). In all cases, flux measurements for each sampling campaign were conducted at 1 h intervals with fluxes being calculated every hour. Mercury captured on gold traps was analyzed for Hg^0 isotopes by ICP-MS as described in the SI.

2.5. Analytical Methods. Detailed information concerning analytical methods are described in the SI, including Hg^0 isotopes captured on gold trap, individual THg isotope analysis of soil samples, and sawgrass plant tissues.

2.6. Quality Control. Quality assurance and control (QA/QC) were carried out throughout the field and laboratory sampling and analysis processes. All flux bag components were rigorously cleaned before use. Before flux measurements, tests were routinely processed to determine the exchange of Hg^0 with surfaces of the empty exposure chamber. This information is available in the SI. The QA/QC data for the isotopic Hg measurement consisted of blanks, triplicate, and the parallel analysis of several certified reference materials as described in the SI.

3. RESULTS AND DISCUSSION

3.1. Absorption, Transport, and Fate of Soil Hg in the Soil−Plant−Air System. *3.1.1. Variations of THg in Soil.* During the growing period, the mean concentrations of ambient THg in soil samples collected from control and treatment groups (Hg isotope spiked in soil) were 253 ± 19 and 269 ± 4.9 $\mu g\ kg^{-1}$, respectively. The concentrations of ambient THg in the soil of control and treatment groups showed a narrow range of variation with time (Figure S3). Similar to ambient THg, the enriched $T^{201}Hg$ in soil samples from the treatment group showed a narrow range of variation with time during the growing period (SI Figure S3). During the growing period, the levels of $T^{201}Hg$ in soil slightly deceased from day 1 (1005 ± 118 $\mu g\ kg^{-1}$) to day 60 (947 ± 67 $\mu g\ kg^{-1}$), indicating that the majority (94%) of the spiked $^{201}Hg^{2+}$ still remained in the soil. Because of the reducing conditions in wet land soil, enriched $^{201}Hg^{2+}$ could be reduced to $^{201}Hg^0$ by biotic or abiotic processes.[12] Moreover, previous studies suggested that wetland plants have the ability to stimulate the release of Hg from soil into pore water and the rhizosphere,[24] which in turn accelerate the reduction of Hg^{2+} in the rhizosphere. Therefore, the slight decrease in $T^{201}Hg$ in soil during the growing period could be attributed to (1) the continual transport of $^{201}Hg^{2+}$ from soil to plant, (2) the emission of formed $^{201}Hg^0$ over soil-air surface,[11] (3) and the adsorption of enriched ^{201}Hg on surface of the experimental pot.

3.1.2. Enriched $T^{201}Hg$ in Tissues of Sawgrass. If root uptake and translocation of Hg was a potential contributing pathway to foliar Hg, the enrichments would be detectable in the plant within the time frame of this exposure study. During the growing period, the average abundance of ^{201}Hg in plant tissues of control groups was 13.35 ± 0.12%, which comparable with the natural abundance of ^{201}Hg (13.2%). The measured abundance of ^{201}Hg in samples from control groups indicates that the control groups was not contaminated with the enriched Hg isotopes during growing period. Our results showed that $T^{201}Hg$ abundance in sawgrass tissues increased above the natural abundances of corresponding individual Hg isotopes throughout the 60 days of exposure (Figure 1A, B), indicating that net accumulation was occurring.

Concentrations of enriched $T^{201}Hg$ in live leaves increased during the growing period from day 1 (5.9 ± 1.9 $\mu g\ kg^{-1}$) to day 30 (10 ± 2.0 $\mu g\ kg^{-1}$) (Figure 1A). The enrichments of $T^{201}Hg$ in leaves at day 1 indicated that soil spiked ^{201}Hg was translocated to the aboveground parts from root uptake and that root uptake of enriched $T^{201}Hg$ was likely a potential source of foliar $T^{201}Hg$ under these experimental conditions. Using a stable isotope tracer technique, Mao et al.[17] suggested that on average 7.4 ± 3.7% of the THg in sawgrass leaves were estimated to be from soil, which supports our results. Enriched $T^{201}Hg$ concentrations in senescing leaves showed a consistent increase from day 1 (5.0 ± 1.2 $\mu g\ kg^{-1}$) to day 60 (12 ± 1.1 $\mu g\ kg^{-1}$) after application. Live leaves showed a decrease in enriched $T^{201}Hg$ concentration at day 60 and enriched $T^{201}Hg$ concentrations in senescing leaves were significantly higher than in live leaves at day 60 ($p < 0.01$) (Figure 1A), probably due to some live leaves becoming senescing leaves. Furthermore, the continual increase and relative higher concentrations of enriched $T^{201}Hg$ in senescing leaves suggested that almost all enriched ^{201}Hg was still stored in leaves over the course of the experiment (see details in section 3.1.3)

Mean concentrations of enriched $T^{201}Hg$ in roots (419 ± 147 $\mu g\ kg^{-1}$) and stems (152 ± 96 $\mu g\ kg^{-1}$) during the growing period were significantly ($p < 0.001$) higher than in live (8.0 ± 3.5 $\mu g\ kg^{-1}$) or senescing (7.6 ± 3.8 $\mu g\ kg^{-1}$) leaves. It seemed that, while root uptake of soil Hg and transportation to aboveground parts did occur, this uptake presented a limited contribution to the concentrations of Hg in leaves.[17] In contrast to the enriched $T^{201}Hg$ in aboveground parts (levels), enriched $T^{201}Hg$ concentrations in belowground parts (root and stem) remained high during the entire growing period, slightly

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decreased in root, and slightly increased in stem from day 1 to day 60. The highest concentrations of enriched $T^{201}Hg$ were always found in the roots, supporting the hypothesis of the roots acting as a barrier for the transport of inorganic Hg.[25,26]

3.1.3. Relative Distribution of Enriched $T^{201}Hg$ among Tissues of Sawgrass. The mass and relative distributions of enriched $T^{201}Hg$ in different tissues of sawgrass during the growing period are shown in Figure 1B. The mass of the enriched $T^{201}Hg$ in leaves (senescing and live leaves) slightly increased from 11 ng plant^{-1} at day 1 to 17 ng plant^{-1} at day 30 and to 21 ng plant^{-1} at day 60 during the growing period, indicating the translocation and accumulation of enriched $T^{201}Hg$ in aboveground parts of sawgrass over time. Compared to leaves, the mass of the enriched $T^{201}Hg$ sharply increased during the period from day 1 to day 30 in roots and from day 1 to day 60 in stems. The mean (range) total mass of enriched $T^{201}Hg$ in the whole plant were 228 ± 93 ng plant^{-1} (120−292 ng plant^{-1}) at day 1, 318 ± 55 ng plant^{-1} (261−371 ng plant^{-1}) at day 30, and 318 ± 135 ng plant^{-1} (184−453 ng plant^{-1}) at day 60, respectively. As shown in Figure 1B, the total mass of enriched $T^{201}Hg$ increased from day 1 to day 30 and then remained unchanged until day 60. The percentage of enriched $T^{201}Hg$ allocated to the aboveground parts slightly increased from day 1 (5.3 ± 2.7%) to day 60 (6.9 ± 1.5%), whereas the percentage of enriched $T^{201}Hg$ in roots decreased from day 1 (87 ± 3.2%) to day 60 (77 ± 3.0%). When harvested (day 60), the majority of the enriched $T^{201}Hg$ accumulated was localized to the belowground parts (93 ± 1.5%), whereas only 6.9 ± 1.5% was translocated to the aboveground parts. Our results showed that even sawgrass took up enriched $T^{201}Hg$ from soil via roots, the absorbed enriched $T^{201}Hg$ was not translocated from roots to leaves in any significant amount relative to the storage of enriched $T^{201}Hg$ in the root zone. In this study, enriched ^{201}Hg was spiked into soil as inorganic $^{201}Hg^{2+}$. Indeed, the sawgrass root could act as a barrier against inorganic Hg entering the interconnected cytoplasms (symplast) of the plant.[25,26] The relative importance of root accumulation of enriched $T^{201}Hg$ suggests that a portion of the THg stored in soil might end up in belowground components of the ecosystem. Roots, therefore, are the main tissue of sawgrass that accumulates Hg relative to the aboveground parts and consequently plays an important role in the fate of Hg and affects its mobility in wetland ecosystems.

3.1.4. Fluxes of Enriched $^{201}Hg^0$ over Sawgrass Leaves and Air. Temporal variations of the hourly surface Hg^0 flux over sawgrass leaves and incoming solar radiation in treatment groups are shown in Figure 2. Ambient gaseous Hg^0 fluxes over sawgrass leaves fluctuated from −42 to 23 ng m^{-2} h^{-1} with an average value of −0.27 ± 11 ng m^{-2} h^{-1} during the entire growing period. The data indicated that Hg^0 exchange over the air-foliar surface was bidirectional and highly variable, but the net flux was primarily characterized by Hg^0 deposition from the atmosphere to sawgrass leaves. Zhang et al.[27] reported in situ Hg fluxes with deposition being dominant when measured from a wetland plant species using a bag method in the field. The implications of these findings are that plants may serve as a conduit for Hg from air pools into plant foliage and thus as a new Hg source to soil in the Everglades.[27] There were, however, other studies that measured greater emission and lower deposition of Hg between air and foliar surfaces.[11−13] These authors suggested that Hg^0 emission from vegetation would result in the Everglades being a net source of Hg to the atmosphere.[11,12] Disparities between our data and the previous

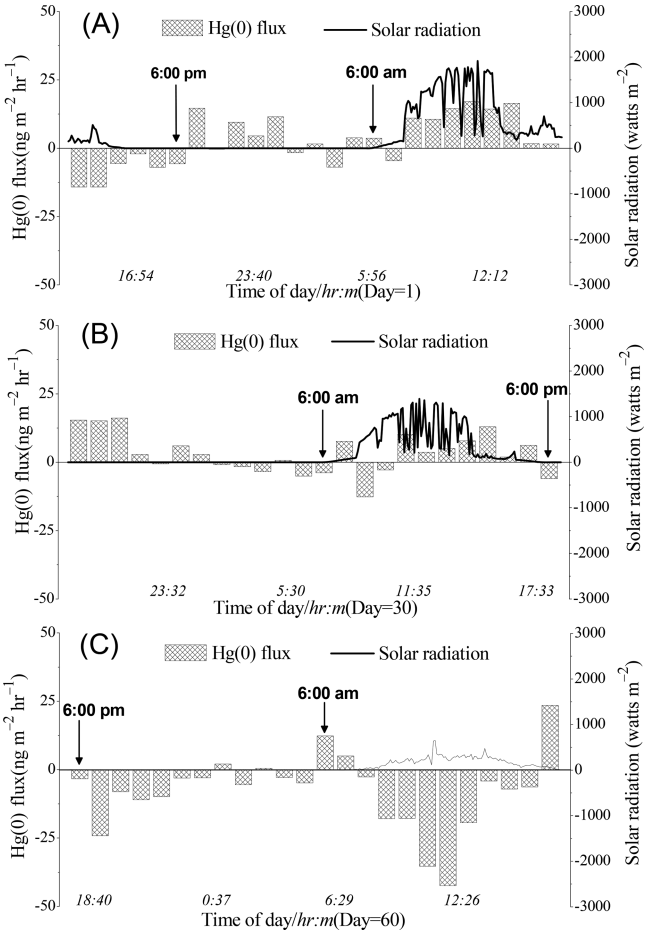

Figure 2. Temporal variations over 24 h of the hourly surface Hg^0 flux over sawgrass leaves (bars) and incoming solar radiation (line) in treatment groups of soil $^{201}Hg^{2+}$ spiking experiments at days 1 (A), 30 (B), and 60 (C).

studies might be related to the differences in plant species, methodology, and soil/air Hg concentrations.

Because the gas-exchange system employed in previous studies detected only the net direction of Hg flux,[28] it was impossible to distinguish the relative contributions of soil vs atmospheric sources in the emission process. Using the Hg^0 flux bag together with isotopically enriched $^{201}Hg^{2+}$ spiked in the soil, we were currently quantifying the fate of spiked ^{201}Hg from the soil, which was uptaken by the roots and transported to aboveground parts of sawgrass on an ongoing basis to directly quantify the emission of Hg from leaves to air in the chamber. In the case of sawgrass, the observed Hg^0 emission from sawgrass leaves to the air raised the question whether the emitted Hg was taken up by plants from soil or it was previously deposited atmospheric Hg^0.

Over the entire growing period, the concentrations of enriched $T^{201}Hg$ in live leaves (7.9 ± 3.5 μg kg^{-1}) were significantly elevated, which were comparable to the ambient THg concentrations observed in either the control (4.9 ± 0.40 μg kg^{-1}) or treatment groups (7.2 ± 1.2 μg kg^{-1}) (SI Figure S4). As detecting spiked Hg usually becomes possible when it is present at a concentration >1% of the ambient Hg^0, this level of enriched ^{201}Hg was enough to detect Hg^0 exchange over the air−foliar surface by using stable Hg tracer technique.[29]

A significant amount of Hg^0 exchange over the foliar−air surface occurred via stomatal and/or nonstomatal pathways

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Table 1. Hg Isotope Ratios in Inlet and Outlet Airstreams of the Flux Bag over Time for Soil and Atmosphere Hg Isotopes Spike Experiments (mean ± SD, range; n = 3)

	$^{201}Hg/^{202}Hg$		$^{199}Hg/^{202}Hg$	
growing periods (day)	inlet	inlet	inlet	outlet
1	0.45 ± 0.01 (0.44−0.46)	0.45 ± 0.01 (0.44−0.46)	0.57 ± 0.01 (0.55−0.58)	0.64 ± 0.04 (0.59−0.74)
30	0.44 ± 0.01 (0.43−0.45)	0.44 ± 0.01 (0.43−0.45)	0.56 ± 0.003 (0.55−0.57)	0.56 ± 0.01 (0.55−0.57)
60	0.44 ± 0.01 (0.42−0.45)	0.44 ± 0.01 (0.42−0.45)	0.56 ± 0.01 (0.55−0.57)	0.56 ± 0.01 (0.54−0.57)

(Figure 2). If the reduction of $^{201}Hg^{2+}$occurred on foliar surfaces and the resulting $^{201}Hg^0$ was emitted to the air, we would observe the enriched $^{201}Hg^0$ in the outlet airstream of the flux bag. However, no discernible difference in the Hg isotope ratio ($^{201}Hg/^{202}Hg$) was observed between the inlet and outlet airstreams of the flux chamber (Table 1). It appeared that even if uptake of spiked ^{201}Hg from soil and transportation from roots to leaves had occurred, no subsequent emission of enriched ^{201}Hg was detectable in the outlet airstream of the flux chamber in either light or dark. This result suggests that the spiked Hg supplied to sawgrass leaves from soil could be incorporated into leaf tissue without being available for re-emission into the atmosphere.[30] Previous study indicated that reduction of Hg^{2+} can occur in transgenic tobacco engineered to express bacterial native mercuric reductase (*MerA*) that facilitates the transport of ionic Hg to cells and the release of Hg^0 to air.[31] It is unclear why Hg accumulated in the leaves of sawgrass from soil cannot be re-emitted to the atmosphere in this study, but we speculate that the absence in reduction pathways of Hg^{2+} present in sawgrass could be one of the possible reasons. If ambient Hg in soil behaves like the experimentally spiked Hg, some of the Hg deposited into the wetland soil in wet deposition would be unavailable to pass through the air−vegetation surface. As a result, the contribution of Hg vapor emission by way of flux processes between air and vegetation into the atmosphere could be smaller than previously thought.[4]

3.2. Absorption, Transportation, and Fate of Air Spiked $^{199}Hg^0$ over the Plant−Air System. *3.2.1. Absorption and Re-emission of Enriched $^{199}Hg^0$ over the Leaf−Air Surface.* In the experiments of air spiked $^{199}Hg^0$, our flux measurements were successful in tracing the processes of Hg^0 flux at the foliar−air surface, including Hg^0 deposition in leaves and Hg^0 re-emission to air (Figure 3). During the 24 h exposure period, additions of $^{199}Hg^0$(g) from the vapor source led to enrichment in the spiked ^{199}Hg isotope from 66 to 123 ng m^{-3}, with a mean concentration of 81 ± 19 ng m^{-3}. At higher atmospheric spiked $^{199}Hg^0$ concentrations, the enriched $^{199}Hg^0$ fluxes varied from 12 to −251 ng m^{-2} h^{-1} with an average value of −77 ± 76 ng m^{-2} h^{-1}. The mean flux of enriched ^{199}Hg was primarily characterized by negative values, indicating that the dominant process for sawgrass was deposition. The re-emission of predeposited enriched ^{199}Hg from sawgrass leaves (0.59 to 12 ng m^{-2} h^{-1}) was observed simultaneously but was of limited significance when compared with the deposition (Figure 3A).

If previously deposited Hg was a significant pathway for Hg re-emission to air, the isotope ratios ($^{199}Hg/^{202}Hg$) in the airstreams between the flux bag inlet and outlet would be different and detected. As shown in Table 1, enriched $^{199}Hg^0$ ratios in inlet and outlet airstreams of the flux bag at day 1 differed from those at day 30 and day 60. The isotope ratios ($^{199}Hg/^{202}Hg$) in the outlet airstream of the flux bag were significantly higher than those in the inlet airstream in the day

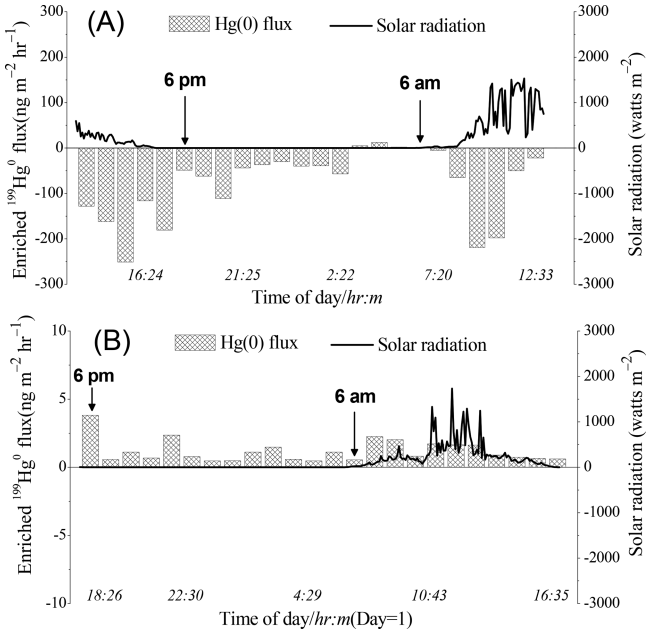

Figure 3. Temporal variation over 24 h of the hourly surface spike $^{199}Hg^0$ flux over sawgrass leaves (bars) and incoming solar radiation (lines) during the 24 h exposure (A) and at day 1 following the 24 h exposure to $^{199}Hg^0$ (B).

following the 24 h exposure (p < 0.001) (Table 1). The only source of the released enriched ^{199}Hg was atmospheric spiked $^{199}Hg^0$ deposited on sawgrass leaves during the exposure experiment. This is strong evidence that the previously deposited ^{199}Hg in leaves can be re-emitted from foliage to air after the $^{199}Hg^0$ exposure. The re-emission of the predeposited Hg increases the residence time of Hg in the atmosphere, enhancing its capability for long-range transport. There was no statistical difference for the $^{199}Hg/^{202}Hg$ ratios between outflows and inflows of the flux bag at days 30 and 60 (Table 1), indicating that no enriched ^{199}Hg was detected in the outflow of the flux bag.

After $^{199}Hg^0$ was applied, the highest enriched ^{199}Hg re-emission (3.8 ng m^{-2} h^{-1}) from leaves was observed during the subsequent hour at ambient air Hg^0 concentrations (Figure 3B). Enriched ^{199}Hg re-emission declined rapidly over the next few hours and leveled off to near the detection limit by the end of the experiment. Leaf−air Hg^0 exchange has been suggested to occur through stomatal[32] and/or a nonstomatal (cuticular) pathways.[28] Therefore, we speculated that the predeposited enriched ^{199}Hg could be temporarily stored in the leaf stomata, and then re-emitted to air over the foliar−air surface. In contrast to the rapid initial re-emission of enriched ^{199}Hg at day 1, no re-emission was observed over the foliar-air surface for flux measurements at days 30 and 60, implying that the majority of surface deposited ^{199}Hg could be retained, possibly being incorporated into epidermal and stomatal cell walls, and

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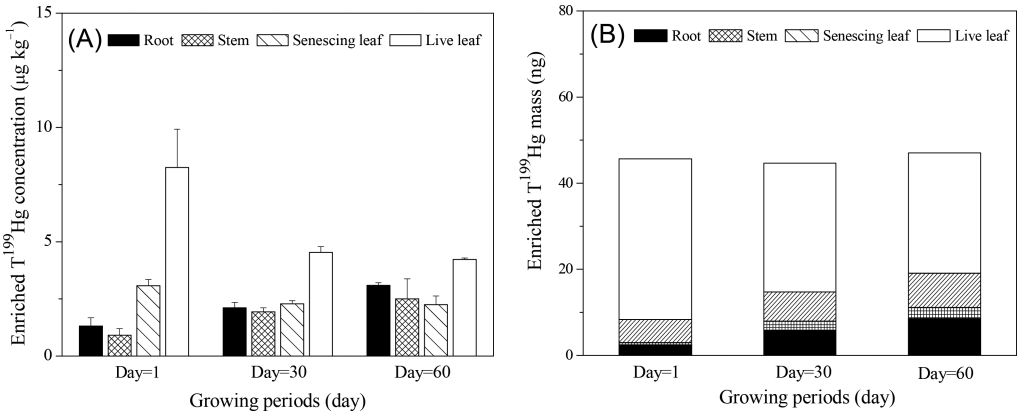

Figure 4. Concentrations (A, n = 3) and relative distributions (B) over time ofenriched^{199}Hg in sawgrass tissues.

parenchyma cell nuclei.[25,33] However, the data supporting this hypothesis are limited. Thus, to better understand this process, further work needs to be done.

Using the concentrations of spiked $^{199}Hg^0$ in the inlet and outlet airstreams of the flux bag, the mass of the spiked $^{199}Hg^0$ deposited in the sawgrass plant over 24 h exposure was calculated in this study (see detail in the SI). On the basis of the enriched $^{199}Hg^0$ flux over the air-foliar surface, we further calculated the release of enriched ^{199}Hg mass from foliar surfaces on the subsequent day. Again, our observation confirmed that no re-emission was observed over the foliar−air surface for flux measurements at days 30 and 60. Therefore, we assumed that re-emission of the spiked $^{199}Hg^0$ from leaves occurred in the subsequent 24 h after the exposure experiment. Our calculated data showed that the mass of the net deposited $^{199}Hg^0$ to foliage enclosed in the flux bag over the 24 h exposure was 45 ng. The corresponding release of enriched ^{199}Hg mass from foliar surfaces during on the subsequent 24 h was 0.71 ng. The re-emitted ^{199}Hg (0.71 ng) accounted for only1.6% of the total deposited ^{199}Hg (45 ng). The relatively small re-emission of $^{199}Hg^0$ from the leaf surface to the air implied that the majority (98.4%) of the taken up $^{199}Hg^0$ in leaves could be fixed and incorporated in leaf tissue and not available for re-emission. The percentage of $^{199}Hg^0$ (f_{fixed} = 98.4%) fixed into tissues through atmospheric dry deposition is much higher than the observations in a previous study (25%).[18] It is worth mentioning that the current study first provides the direct evidence to trace the deposition and re-emission processes of atmospheric Hg^0 exchange over the air−foliar surface. Our results will help reduce the model uncertainty in estimating the global and/or regional natural Hg^0 exchange over the vegetation surface. However, it should be noted that our study was just focused on the typical wetland plant (sawgrass), and the results might not be directly applicable to other vegetation surfaces.

3.2.2. Location and Transportation of Hg Taken up from Air within the Sawgrass Plant. The location and transport of the enriched ^{199}Hg over time in the sawgrass were determined in this study (Figure 4). Foliar (live leaf) concentrations of enriched T^{199}Hg showed a consistent decline from day 1 to day 60 (Figure 4A). Most of the decrease in foliar enriched T^{199}Hg concentration occurred within the first 30 days, and after this initial period, concentrations decreased much more slowly. Enriched T^{199}Hg concentration in new foliage (live leaf) (8.3 ± 1.7 μg kg^{-1}) was approximately 3 times higher than in the old leaves (senescing leaf) (3.1 ± 0.27 μg kg^{-1}) at day 1. Since transpiration, photosynthesis, and growth rates are related to stomatal conductance and typically greater for new leaves than for old leaves, stomatal conductance may be an important parameter controlling Hg^0 uptake. This, along with the higher enriched T^{199}Hg concentrations observed in new leaves, suggests that increased stomatal conductance in sawgrass plants may facilitate atmospheric $^{199}Hg^0$ uptake. Previous studies observed that conductance rates and photosynthetic rates decreased as a function of leaf age; these researchers further suggested that younger tissues present the strongest accumulation of Hg^0 vapor, with accumulation slowing down and leveling off as tissues age.[34,35] The occurrence of enriched T^{199}Hg in senescing or senescent leaves on day 1 indicated that the old leaves were still functional for uptake of $^{199}Hg^0$ during the exposure experiments. The relatively low concentrations of enriched T^{199}Hg in senescing or senescent leaves suggest that old leaves could accumulate less $^{199}Hg^0$ through their stomata as the leaves stop growing.

Enrichment of T^{199}Hg in both sawgrass stem and root was detected on day 1, but at a lesser degree than those found in leaves. As shown in Figure 4A, enriched T^{199}Hg concentrations in stem and root increased over the course of the experiment (60 days). The only route leading to the increase of enriched T^{199}Hg was through leaf-stem-root transport. Hence, we suggest that spiked $^{199}Hg^0$ in air was taken up by the leaves and translocated from the foliage to other parts of the plant, such as stem and root. This is the first direct evidence that sawgrass is capable of moving Hg from the aboveground parts to the belowground parts. However, the mechanism of this translocation process in sawgrass plant is still unclear.

To determine how much enriched $^{199}Hg^0$ accumulated in the sawgrass plant over the 24 h exposure, we calculated the enriched T^{199}Hg uptake in the whole plant during the growing period (Figure 4B). Approximately 46 ng of enriched T^{199}Hg was retained in the whole plant at day 1. The accumulation calculated here was well in agreement with that calculated for spiked $^{199}Hg^0$ deposition (45 ng) based on foliar $^{199}Hg^0$ fluxes over the 24 h exposure. At day 1, a predominant fraction (93%) of the enriched T^{199}Hg uptake from air was in the leaves (live leaf = 81%; senescing or senescent leaf = 12%). Accumulation in stem (1.0%) and root (6.0%) was much less significant. However, at the end of the experiment (Day = 60), the percentage of enriched T^{199}Hg in leaves (76%) was significantly decreased; accumulation was shifted to 5.0% and 18% in stem and root, respectively, further confirming the continuous translocation of enriched T^{199}Hg from aboveground to

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belowground parts of sawgrass. During the growing period, the percentage of the enriched $T^{199}Hg$ showed a slight decrease in live leaves but a slight increase in senescing leaves (Figure 4B), probably due to some live leaves becoming senescing leaves and thus the translocation of enriched $T^{199}Hg$ from new to old leaves.

No significant difference among the three sampling campaigns ($p > 0.05$) was observed for the total mass of enriched $T^{199}Hg$ in the whole plant, meaning that almost all of the enriched ^{199}Hg uptaken from air was still stored in the sawgrass tissues. Furthermore, the enriched $T^{199}Hg$ accumulation in plants over the 60-day period was compared to the re-emission of the enriched $^{199}Hg^0$ during the subsequent 24 h following exposure. Although the enriched $^{199}Hg^0$ accumulated in leaves can be re-emitted to the air during the first day, the leaf-re-emitted $^{199}Hg^0$ (0.71 ng) only accounted for 1.5% of the enriched $T^{199}Hg$ retained in the whole plant at day 1. Such observations suggest that the majority of the previously deposited $^{199}Hg^0$ taken up by leaf was fixed, with a very limited amount available for rapid exchange between the leaf and atmosphere.

3.3. Fate of Hg in Sawgrass and Potential Environmental Implications. This work reported experimental investigations on the role of sawgrass in biogeochemical cycling of Hg in the Everglades. The behavior of foliar-exposed $^{199}Hg^0$, together with the observations of soil spiked $T^{199}Hg$ located in sawgrass tissues, suggests that net Hg^0 emission from the sawgrass leaf could be attributed to previous Hg deposition on the foliage. A significant portion of foliar-deposited $^{199}Hg^0$ could be rapidly oxidized and fixed in the foliage, whereas only a minor portion was re-emitted to the air through foliar−air flux during the entire growing period. Recently, Mao et al.[17] found that at most 7.4 ± 3.7% of Hg in sawgrass leaves originated from soil uptake by the roots and the majority (>90%) of uptake was from atmospheric sources. The current study, agreeing well with the previous work,[17] emphasizes that sawgrass foliage needs to be viewed as an important sector for atmospheric Hg deposition. When leaves senesce and fall off the sawgrass plant, Hg retained in litterfall represents a new input of fixed atmospheric Hg to the Everglades. As leaf litter is decomposed, fixed Hg can be redistributed into the soil. Hall and St. Louis[36] observed that Hg in litterfall could be methylated even on dry soils, suggesting that litterfall may be a source of MeHg to wetland ecosystems. The results of the present investigation did not agree with previous studies by Lindberg et al.,[11,12] where it was found that Everglades leaf surfaces acted as biogenic sources of Hg vapor for ambient air. Because gas-exchange systems measure only the net direction of Hg flux, previous studies could not distinguish the relative contributions of soil vs atmospheric sources of Hg^0.

This work presents important environmental implications on the biogeochemical cycling of Hg in Everglades. If ambient Hg^0 in the air interacts with foliage like our experimentally applied spiked $^{199}Hg^0$, the majority of the Hg^0 taken up by sawgrass leaves would be unavailable for re-emission to the air. As a result, atmospheric Hg provided a substantial flux of Hg^0 via stomatal and/or nonstomatal absorption when compared with inputs from direct soil uptake and transportation. This suggests that the input of Hg into the Florida Everglades by sawgrass leaves could be larger than previously estimated.[4] Uptake of the atmospheric Hg^0 reservoir by sawgrass leaves could substantively increase the Hg load, and thus it is important to evaluate the Hg input through litterfall in order to fully understand the biogeochemical cycling of Hg in the Florida Everglades.

We demonstrate in this study the relative importance of sawgrass as receptors for Hg in wetland ecosystems and suggest that sawgrass leaves must be considered a sink for atmospheric Hg in the regional Hg mass balance. This information is useful for accurately quantifying the critical loads of Hg to the Everglades, as currently direct wet deposition is often considered as the only major Hg input to the ecosystem.[4] Furthermore, our study provides useful information for creating models of natural deposition of Hg and fills gaps in our understanding of the role of wetland vegetation in the biogeochemical cycling of Hg in the Everglades. The data processing techniques employed here for constructing a mass inventory and mass budget of Hg could be applied to other ecosystems.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b04150.

Measurement of Hg flux over the leaf−air surface; quantifying the mass of enriched $^{199}Hg^0$ taken up by sawgrass and the re-emission of the ^{199}Hg to the atmosphere; sample collection and preparation; protocol for individual THg isotope analyses in soil and plant samples; the QC/QA protocol and statistical analysis of analytical data; the biomass of sawgrass plants in different groups; 2 Tables; 4 Figures (PDF)

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Notes

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