Nitrogen Source Tracking with δ^{15}N Content of Coastal Wetland Plants in Hawaii

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Inter- and intra-site comparisons of the nitrogen (N) stable isotope composition of wetland plant species have been used to identify sources of N in coastal areas. In this study, we compared δ^{15}N values from different herbaceous wetland plants across 34 different coastal wetlands from the five main Hawaiian Islands and investigated relationships of δ^{15}N with land use, human population density, and surface water quality parameters (i.e., nitrate, ammonium, and total dissolved N). The highest δ^{15}N values were observed in plants from wetlands on the islands of Oahu (8.7–14.6‰) and Maui (8.9–9.2‰), whereas plants from wetlands on the islands of Kauai, Hawaii, and Molokai had δ^{15}N values usually <4‰. The enrichment in δ^{15}N values in plant tissues from wetlands on Oahu and Maui was most likely a result of the more developed and densely populated watersheds on these two islands. Urban development within a 1000-m radius and population density were positively correlated to average δ^{15}N vegetation values from each wetland site ($r = 0.56$ and 0.51, respectively; $p < 0.001$). This suggested that site mean δ^{15}N values from mixed stands of wetland plants have potential as indices of N sources in coastal lowland wetlands in Hawaii and that certain sites on Oahu and Maui have experienced significant anthropogenic N loading. This information can be used to monitor future changes in N inputs to coastal wetlands throughout Hawaii and the Pacific.

Naturally occurring stable N isotopes have been successfully used to identify sources of N in coastal ecosystems, especially when sources have distinct δ^{15}N values (McClelland et al., 1997; McClelland and Valiela, 1998; Cole et al., 2004). Dissolved inorganic nitrogen (DIN) inputs to watersheds such as precipitation (δ^{15}N of −7‰ to +1‰), biologically fixed N (δ^{15}N ~ 0‰), and commercial inorganic fertilizers (δ^{15}N of −3‰ to +3‰) have lighter δ^{15}N values compared with human waste water and livestock waste (+4 to +6‰) (Macko and Ostrom, 1994; McClelland et al., 1997; McClelland and Valiela, 1998; Kjønas and Wright 2007; Barnes et al., 2008). As these different DIN pools move through watersheds, their δ^{15}N values can become enriched through disproportionate loss of the lighter ^{14}N isotopes via ammonia volatilization or denitrification (Cifuentes et al., 1989; Macko and Ostrom, 1994). Although this can result in similar δ^{15}N values among pools (e.g., fertilizer-derived vs. rain-derived DIN), these processes can also significantly enrich the δ^{15}N values of residual N derived from sewage and livestock waste such that their δ^{15}N values (+10 to +38‰) are distinct from other DIN sources (Macko and Ostrom, 1994; Savage, 2005). As N contributions to vegetation from sewage increase, the δ^{15}N values of plants can become more enriched. In contrast, as N contributions to vegetation from inorganic fertilizers or precipitation increase, the δ^{15}N values of plants can become less enriched (Wigand et al., 2007).

In the northeastern USA, δ^{15}N values of smooth cordgrass (*Spartina alterniflora* Loisel), a common salt marsh species, have been shown to be effective indicators of watershed N sources in coastal areas (McClelland et al., 1997; McClelland and Valiela, 1998; Wigand et al., 2001; Wigand et al., 2007). Other salt marsh species, such as Jesuit's bark (*Iva frutescens* (L.)), common reed (*Phragmites australis* (Cav.)), and saltmeadow cordgrass (*Spartina patens* (Ait.) Muhl) have also been used as indicators of N sources (Wigand et al., 2007). Other researchers have investigated δ^{15}N values of sawgrass (*Cladium jamaicense* Crantz) and cattail (*Typha domingensis* Pers.) tissue to investigate patterns of nutrient enrichment and eutrophication in subtropical Everglades wetlands (Inglett and Reddy, 2006; Chang et al., 2008). Thus, the δ^{15}N values of wetland plants can help coastal zone managers monitor changes in inputs of N (McClelland and Valiela, 1998).

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Abbreviations: BDL, below detection limit; δ^{15}N, delta 15 nitrogen isotopic content; DIN, dissolved inorganic nitrogen; MSAL, Marine Sciences Analytical Laboratory; NH_4–N, ammonium-nitrogen; NO_3–N, nitrate-nitrogen.

Unfortunately, only two of the plant species listed above (sawgrass and cattail) are found in coastal wetlands of Hawaii, and they are only minor components in terms of cover and biomass in most Hawaiian and Pacific Island wetlands. Plant community structure in Hawaii also differs considerably among wetlands, which is largely a result of the isolated nature of Hawaii's wetlands and the introduction of exotic plants (Bantilan-Smith et al., 2009). As a result, intersite comparisons of the same plant species can be difficult if not impossible because one species does not occur across all coastal wetland sites.

Thus, the use of wetland plant tissue δ^{15}N as an indicator of N sources needs to continue to be tested in estuaries with different land uses and in different climatic conditions (McClelland and Valiela, 1998). Furthermore, most studies have compared δ^{15}N values of individual plant species across different sites. Few studies have examined the utility of δ^{15}N values of multiple species to track sources of N, and none has been conducted on Pacific Islands, where increasing human population densities and development threaten coastal ecosystems (Laws and Ferentinos, 2003).

We attempted to determine if the δ^{15}N values of mixed plant communities could function as indicators of N sources when compared across different wetlands on the five main Hawaiian Islands. The specific objectives of this study were: (i) to evaluate differences in coastal wetland plant δ^{15}N values across wetland sites located in watersheds of different land-use/land-cover and (ii) to investigate relationships between δ^{15}N values of coastal wetland plants and land use, demographic data (i.e., population density), and surface water quality parameters (i.e., nitrate, ammonium, total dissolved N). Results from this study will facilitate comparisons of δ^{15}N values between coastal wetlands in more-developed versus less-developed watersheds in Hawaii and provide baseline monitoring of N stable isotope values in coastal wetland plants. These values can serve as points of comparison for future studies and allow for the quantification of changes in N sources in these watersheds over time (McClelland et al., 1997).

Materials and Methods

Study Sites

Thirty-four coastal lowland wetland sites across the state of Hawaii were sampled between March and April 2007 (Fig. 1). To restrict our focus to coastal lowland wetlands on the main Hawaiian Islands, we used the following criteria: (i) Sites were located between 0 and 100 m in elevation and (ii) sites were located on one of the five major Hawaiian Islands (Hawaii, Kauai, Maui, Molokai, and Oahu). The elevation criterion effectively excluded all mountain bogs, which comprise a significant component of Hawaii's wetland area but are considerably different in structure and function than coastal lowland wetlands. Because various coastal wetland sites are located on private property or on military land with restricted access, a random sampling of all coastal wetlands was impossible. Efforts were made to achieve balance between isolated and riparian/estuarine sites and among fresh water, brackish, and euhaline sites. In sampling 34 wetlands, we are confident that our sites represented the overall population of coastal wetlands in Hawaii. Site ownership varied from federal (U.S. Fish and Wildlife Service Refuges, National Historic Parks, and Marine Corps Base Hawaii) to state (Hawaii Department of Land and Natural Resources), county (Hawaii County), non-governmental organizations (Maui Coastal Land Trust and National Tropical Botanical Garden), and private lands.

Vegetation Sampling

Leaf or blade samples were collected from the three most dominant (based on visual estimates of cover) herbaceous plant species (i.e., grasses, sedges, other monocots, dicot herbs) at each wetland site. Grasses and sedges were distinguished from other monocots based on morphological characteristics (e.g., flower arrangement) as described in Erickson and Puttock (2006). At sites where less than three plant species were present, we collected as many species as possible. For grasses, the external-most blade was collected by clipping the blade off close to the root. For monocots and dicots, leaf samples furthest from the apical meristem were clipped from the stem at the leaf petiole. For sedges, culms were sampled. We collected samples of each species from three different individual plants, which were then composited to reduce the effects of intraspecific variability in δ^{15}N. This resulted in the collection of 70 composite plant samples from 34 sites. The samples were placed into sterile whirl pack bags, immediately stored on ice, and returned to the laboratory for processing within 1 to 3 d. Native versus introduced species status was determined based on Erickson and Puttock (2006) and Starr and Starr (2007).

Surface Water Quality Sampling

Physical and chemical properties of surface water were sampled at two locations at each site. The two locations were randomly selected from four representative sites (i.e., hydrology, vegetation, and soils characteristic of the site) identified from aerial images or initial reconnaissance surveys. A handheld multiprobe system (Model 556; YSI Inc., Yellow Springs, OH) was used to measure temperature, dissolved oxygen, pH, conductivity, and salinity. Two 50-mL surface water samples were collected in polypropylene centrifuge tubes at each sampling location. Samples were filtered with a 0.45-μm nylon syringe-tip filter. All samples were frozen and transported as soon as possible (<14 d) to the Marine Science Analytical Lab (MSAL) at University of Hawaii Hilo for analyses. At the MSAL, samples were thawed and analyzed for nitrate-nitrogen (NO_3–N) (USEPA Method 353.2 [USEPA, 1983]), ammonium-nitrogen (NH_4–N) (USGS Method I-2525–89 [Fishman, 1993]), and total dissolved nitrogen (TDN) (ASTM Method D5176 [ASTM, 1995]).

Laboratory Analysis of Plant Tissues

Plant tissue samples were washed with deionized water, dried to constant weight at 60°C, ball milled into a fine powder, and packed into 4 × 6-mm tin boats. Samples were analyzed for δ^{15}N using a Costech ECS 4010 elemental analyzer (Costech Analytical Technologies, Inc., Valencia, CA) interfaced with a

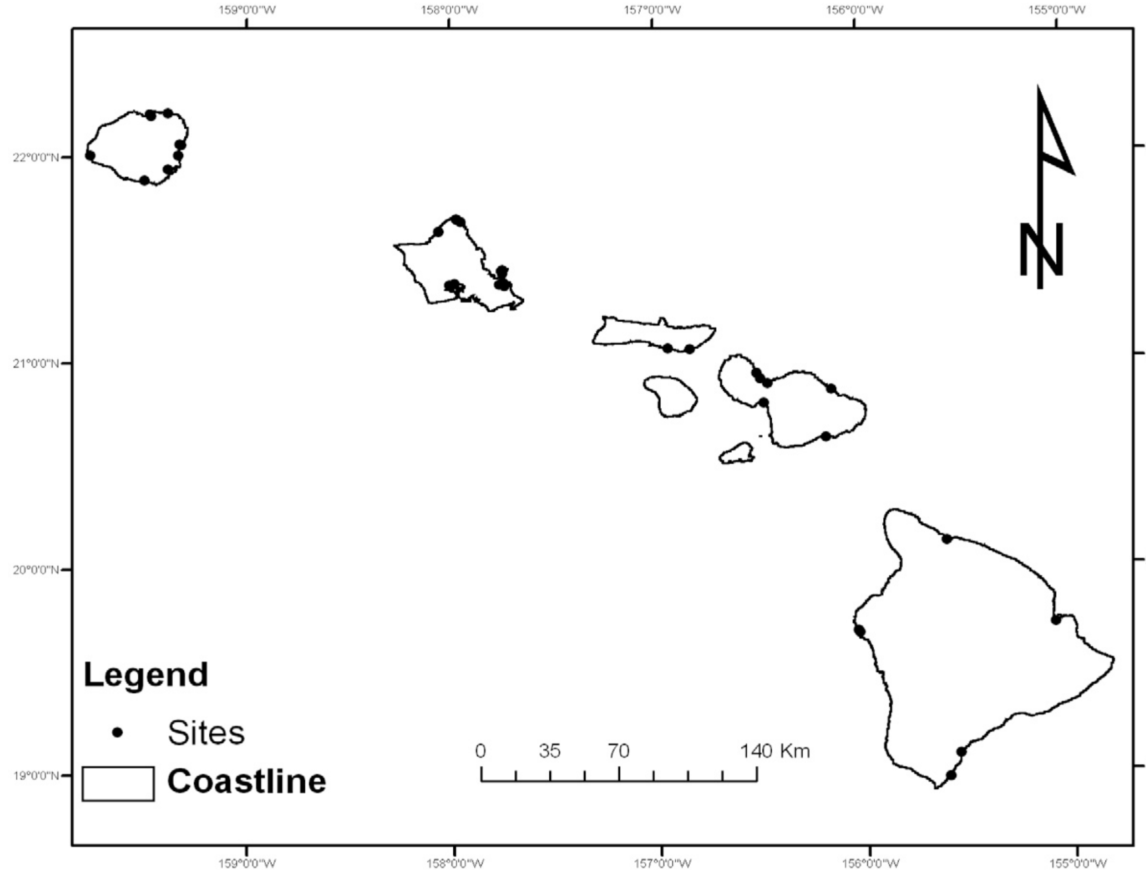

Fig. 1. Locations of the 34 coastal lowland wetlands sites across the five major Hawaiian Islands.

Thermo-Finnegan Delta V Advantage dual isotope mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA) at the MSAL. Stable isotope values were calculated using standard delta (δ) values by the following formula:

$$\delta X = [(R_{\text{samples}}/R_{\text{standard}})\ 1] \times 1000 \qquad [1]$$

where X is ^{15}N expressed in terms of per mil (‰), and R is the corresponding ratio of ^{15}N/^{14}N. Results are presented as deviations from atmospheric N. Internal standards included IAEA N-1, IAEA N-2 (ammonium sulfate), IAEA N-3 (potassium nitrate), USGS-25 (ammonium sulfate), acetanilide, glycine, and peach tree leaves (NIST-1547).

Watershed Land Use and Demographic Determination

The 34 sites sampled were located in 27 different watersheds across Kauai, Oahu, Molokai, Maui, and Hawaii that varied in surface water salinity, hydrology, vegetation, soils, and land use (Tables 1 and 2; Fig. 1). Watershed land use data were obtained from the NOAA Coastal Change Analysis Program (C-CAP). The C-CAP analyses used Landsat 7 Enhanced Thematic Mapper imagery from 2000 to 2001 to map the distribution of land cover types across the state of Hawaii. To minimize cloud cover, multiple image dates and scenes were used to map each island. A band ratio technique was used to mask clouds and cloud shadow areas from the imagery, and cloud-free pieces of overlapping images were then reinserted into the imagery to produce a mosaic with minimal cloud cover. The islands were broken into classes of major land cover features such as developed, cultivated land, grass/scrubland, forest, wetland, bare land, shoreline, and open water. The low- and high-intensity developed categories were combined into a single developed land-use category, and the grassland and scrubland categories were combined into a single grass and scrub category.

A shapefile of watershed boundaries for each of the five islands was obtained from the Hawaii Statewide GIS Program (available at http://hawaii.gov/dbedt/gis/; verified 1 May 2009). Most watershed boundaries were created automatically from smoothed 1:24,000 scale digital elevation models from the USGS. Watersheds that could not be automatically delineated in the GIS were manually digitized on-screen or from paper 1:24,000 scale topographic quadrangle maps. Percent cover was determined for an area radiating 1000 m out from each of the 34 wetland sites in Arc-GIS 9.1 (Environmental Systems Research Institute, 2006).

Areal extent of the different land use types within a 1000-m radius area around each wetland was determined from GIS as described above (Table 2). The 1000-m radius was selected because it proved to be a better predictor of δ^{15}N values according to linear regression than the land use in the contributing watershed, which was also tested. The land uses in the local radius may have performed better than contributing watershed because the radius approach used a similar spatial scale for all sites, whereas the contributing watersheds varied greatly

Table 1. Geographic, physical, vegetative, and environmental characteristics of each of the 34 coastal wetland sites.

Site	Island	Surface water salinity	Hydrology†	Dominant vegetation observed	Species observed (species sampled)‡	Soil order§
Hanalei	Kauai	fresh water	connected	*Urochloa mutica*	5 (3)	I
Huleia	Kauai	fresh water	isolated	*Bacopa monnieri*	11 (3)	I
Kauaihau Co.	Kauai	brackish	isolated	*Paspalum vaginatum*	5 (3)	M
Kauaihau Ri.	Kauai	fresh water	connected	*U. mutica*	6 (3)	I
Kawaiele	Kauai	brackish	isolated	*Sesuvium portulacastrum*	12 (2)	E
Kilauea	Kauai	brackish	connected	*U. mutica*	6 (3)	M
Lawai Kai	Kauai	brackish	connected	*Cyperus javanicus*	10 (3)	I
Nukolii	Kauai	brackish	isolated	*P. vaginatum*	5 (3)	M
Coconut grove	Oahu	brackish	isolated	*Bolboshoenus maritimus*	12 (2)	M
Hamakua	Oahu	brackish	connected	*(B. maritima)*¶, *B. maritimus*	7 (1)	Al
Kaelelpulu	Oahu	brackish	connected	*P. vaginatum*	6 (1)	I
Kawainui	Oahu	fresh water	connected	*U. mutica*	6 (2)	M
Kii	Oahu	brackish	isolated	*(B. maritima)*¶, *C. javanicus*	4 (1)	M
Klipper Pond	Oahu	brackish	isolated	*B. monnieri*	10 (2)	M
Perc. Ditch	Oahu	fresh water	isolated	*B. monnieri*	11 (2)	Ar
Pouhala	Oahu	hyperhaline	isolated	*Carduelis barbata*	4 (1)	I
Punamano	Oahu	freshwater	isolated	*Schoenoplectus spp.*	8 (3)	Coral
Waiawa	Oahu	hyperhaline	isolated	*Panicum maximum*	3 (2)	M
Waimea	Oahu	brackish	connected	*U. mutica*	3 (2)	E
Kakahaia	Molokai	brackish	isolated	*(B. maritima)*¶, *C. papyrus*	3 (1)	Ar
Ualapue	Molokai	euhaline	connected	*U. mutica*	7 (2)	Al
Kanaha	Maui	brackish	isolated	*P. vaginatum*	4 (2)	E
Kealia	Maui	hyperhaline	isolated	*(B. maritima)*¶, *B. maritimus*	3 (1)	Ar
Keanae	Maui	brackish	connected	*U. mutica*	11 (3)	I
Nuu	Maui	brackish	isolated	*B. maritimus*	4 (2)	I
Pakukalo	Maui	fresh water	connected	*U. mutica*	6 (3)	E
Waihee	Maui	brackish	isolated	*P. maximum*	5 (2)	E
Aimakapa	Hawaii	brackish	isolated	*P. vaginatum*	3 (1)	Lava
Honuapo	Hawaii	brackish	connected	*P. vaginatum*	4 (1)	Lava
Kaloko	Hawaii	brackish	connected	*(B. maritima)*¶, *P. setaceum*	4 (1)	Lava
Kamilo Pt. 6	Hawaii	brackish	isolated	*P. vaginatum*	4 (2)	Lava
Kamilo Pt. 7	Hawaii	brackish	isolated	*B. monieri*	4 (2)	Lava
Mohouli	Hawaii	brackish	connected	*Ageratum conzoides*	12 (2)	H
Waipio	Hawaii	fresh water	connected	*Schoenoplectus* spp.	12 (3)	I

† The sites were divided into two groups based on their surface water hydrology: those that were connected (i.e., riparian or tidal) and those that were isolated (i.e., not receiving direct streamflow or tidal inputs) based on Bantilan-Smith et al. (2009).

‡ The number of species observed at each site was based on the sampling described in Bantilan-Smith et al. (2009). The number in parentheses represents the number of species sampled for $\delta^{15}N$ analysis in this study.

§ Al, Alfisol; Ar, Aridisol; E, Entisol; H, Histosol; I, Inceptisol; M, Mollisol. Soils classified as Coral or Lava have yet to be assigned a soil order.

¶ *Batis maritima* was the dominant species at these sites, but it was not included in this study because it is considered a woody herb (Erickson and Puttock, 2006).

in size and in spatial distribution of land uses. Other studies have shown that land use within a localized radius of a wetland site is a better predictor of environmental characteristics such as water quality than watershed level land use (Houlahan and Findlay, 2004). Land uses included development, agriculture, grass/scrub, forest, wetland, bare land, and open water. Demography data were obtained from a 2000 Census Block shapefile available for the Hawaii Statewide GIS Program. Census blocks were clipped to watershed boundaries in ArcGIS, and population density (people ha^{-1}) and total population for each watershed were calculated.

Statistical Analyses

Site means were determined from the $\delta^{15}N$ values from all plants sampled at a site. Pearson's product moment correlation analysis was used to examine relationships among site mean $\delta^{15}N$ values for each of the 34 wetlands and N content (%) of the plant tissue, surface water quality parameters, land-use within a 1000-m radius, and watershed population densities. The $\delta^{15}N$ values from individual plants measured at each of the 34 sites, from individual plant groups (i.e., grasses, sedges, dicot herbs), and from plant species that were present at five or more sites were also analyzed using linear regression to examine relationships among the $\delta^{15}N$ values and land-use data and demographic data. All parametric statistical tests were conducted using SAS for Windows Version 9.1 (SAS Institute, 2002–2003) with a significance level of $p < 0.05$.

Results

Watershed Land Use, Demographics, and Water Quality Data

Mean percent development within a 1000-m radius of the wetland sites was 28.9 ± 8.5% for Oahu, 4.4 ± 2.0% for Molokai, 3.8 ± 0.8% for Kauai, 3.6 ± 1.6% for Hawaii, and 2.4 ± 0.9% for Maui. Percent agriculture within a 1000-m radius was 21.6 ±

Table 2. Watershed demographic characteristics, area, land-use within a1000-m radius, and site mean $\delta^{15}N$ (‰) values for the 34 sampling sites.

Site	Watershed population 2000	Watershed area	Population density	Land-use within a 1000-m radius of wetland site: Developed	Agriculture	Grass and scrub	Forest	Wetland	Shore	Bare	Water	Site mean $\delta^{15}N$
		ha	people ha^{-1}	%								‰
Kauai sites (*n* = 8)												
Hanalei	1069	6127	0.17	0.8	2.3	67.9	25.3	2.4	0.0	0.1	1.2	4.6
Huleia	751	7259	0.10	1.2	4.1	68.4	24.1	1.2	0.0	0.2	0.9	3.0
Kauaihau Co.	4900	1849	2.65	5.3	5.1	56.6	31.2	0.8	0.0	0.1	0.8	2.9
Kauaihau Ri.	4900	1849	2.65	5.3	5.1	56.6	31.2	0.8	0.0	0.1	0.8	3.2
Kawaiele	56	716	0.08	2.6	27.8	62.6	1.6	0.0	0.3	4.7	0.4	3.1
Kilauea	2784	3314	0.84	2.0	4.9	46.7	37.9	7.3	0.1	0.0	1.0	2.8
Lawai Kai	5285	2420	2.18	7.0	15.6	47.7	28.1	0.5	0.1	0.2	0.8	2.6
Nukolii	600	967	0.62	6.0	42.8	38.3	11.7	0.1	0.0	1.0	0.0	4.7
Oahu sites (*n* = 11)												
Coconut Gr.	3231	2713	1.19	4.9	6.7	49.4	33.4	2.5	0.1	1.4	1.7	8.7
Hamakua	26,229	1403	18.70	50.1	0.0	36.0	9.8	0.1	0.0	1.0	3.0	9.5
Kaelelpulu	26,299	1403	18.75	50.1	0.0	36.0	9.8	0.1	0.0	1.0	3.0	7.3
Kawainui	38,739	3806	10.2	27.0	0.0	37.3	15.3	18.7	0.0	0.1	1.4	9.1
Kii	3231	2713	1.19	4.9	6.7	49.4	33.4	2.5	0.1	1.4	1.7	6.5
Klipper Pond	14,931	940	15.88	49.1	0.0	39.4	1.0	0.7	0.4	2.6	6.7	14.7
Perc. Ditch	14,931	940	15.88	49.1	0.0	39.4	1.0	0.7	0.4	2.6	6.7	3.5
Pouhala	118,067	13,427	8.79	65.2	0.0	29.0	0.6	1.6	0.0	3.6	0.0	9.2
Punamano	3231	2713	1.19	4.9	6.7	49.4	33.4	2.5	0.1	1.4	1.7	6.1
Waiawa	33,321	2713	12.28	10.9	0.5	57.6	30.3	0.3	0.0	0.2	0.2	13.1
Waimea	2538	3571	0.71	0.1	1.8	42.4	55.3	0.0	0.1	0.2	0.2	2.6
Molokai sites (*n* = 2)												
Kakahaia	305	3541	0.09	1.9	0.0	84.9	9.6	0.7	0.0	2.7	0.2	4.7
Ualapue	288	503	0.57	3.1	0.4	72.4	22.8	0.3	0.1	0.3	0.7	4.1
Maui sites (*n* = 6)												
Kanaha	42,705	13,326	3.20	4.9	43.5	36.1	3.3	0.0	0.0	11.8	0.4	4.9
Kealia	1697	3667	0.46	1.2	38.1	33.3	17.3	1.5	0.0	7.5	1.2	8.9
Keanae	447	5353	0.08	0.1	0.1	33.5	46.4	0.0	0.0	19.8	0.0	3.5
Nuu	352	5659	0.06	0.2	0.0	59.7	2.4	0.0	0.0	37.5	0.2	9.2
Pakukalo	5501	2687	2.05	4.0	23.8	25.3	44.6	0.0	0.2	1.9	0.1	9.0
Waihee	5501	26,87	2.05	4.0	23.8	25.3	44.6	0.0	0.2	1.9	0.1	6.6
Hawaii sites (*n* = 7)												
Aimakapa	4452	3863	1.15	8.6	0.0	61.1	21.0	0.0	0.0	9.1	0.2	3.7
Honuapo	132	25,364	0.01	0.1	0.1	25.7	50.3	0.0	0.0	23.7	0.0	3.7
Kaloko	6929	3863	1.79	10.3	0.3	44.6	10.2	0.0	0.0	34.4	0.1	1.1
Kamilo Pt. 6	1992	31,145	0.06	0.6	0.5	47.3	31.5	0.0	0.1	19.9	0.1	2.9
Kamilo Pt. 7	1992	31,145	0.06	0.6	0.5	47.3	31.5	0.0	0.1	19.9	0.1	2.1
Mohouli	38,557	48,475	0.80	3.5	0.5	24.6	51.7	0.0	0.0	19.6	0.1	3.9
Waipio	2217	7192	0.31	1.3	0.7	53.2	44.8	0.1	0.0	0.0	0.0	3.7
All sites (*n* = 34)												
Mean	11,278	6536	4	12	7	49	24	1	0	6	1	5.6
SE	3321	1596	1	3	2	2	3	0	0	2	0	3.3
Minimum	56	468	0.01	0	0	25	1	0	0	0	0	1.1
Maximum	118,067	48,475	19	65	44	85	55	7	0	38	7	14.7

7.5% for Maui, 13.5 ± 5.2% for Kauai, 2.2 ± 1.0% for Oahu, 0.4 ± 0.1% for Hawaii, and 0.1 ± 0.1% for Molokai. Mean population density was 9.5 ± 2.6 people ha^{-1} for watersheds on Oahu, 1.3 ± 0.5 people ha^{-1} for watersheds on Maui, 1.2 ± 0.4 people ha^{-1} for watersheds on Kauai, 0.6 ± 0.3 people ha^{-1} for watersheds on Hawaii, and 0.6 ± 0.3 people ha^{-1} for watersheds on Molokai.

Mean surface water salinities were lowest for wetland sites on Kauai and highest for sites on Oahu in the March–April 2007 sampling event (Table 3). The Pouhala site on Oahu had an extremely high mean salinity ($n = 2$) of 138.26‰ in March 2007. Site mean pH values ranged from 6.26 to 9.68 across all islands, with lower mean values for sites on Kauai and higher values for sites on Maui and Molokai. Mean NO_3–N values ranged from below detection limit to 0.99 mg L^{-1}. The highest mean NO_3–N values were observed for sites on Maui, and the lowest values were observed for sites on Molokai. Ammonium-N values ranged from below detection limit to 6.02 mg L^{-1}. The highest mean values were observed for sites on Kauai, and sites on Hawaii had the lowest values. Total dissolved N values ranged from 0.04 to 5.11 mg L^{-1}, with higher values on Oahu, Molokai, and Maui, and considerably lower values on Kauai and Hawaii.

Plant Tissue $\delta^{15}N$ among Species and Sites

The 70 composite plant tissue samples comprised 21 different plant species, four of which were native and 17 of which were introduced (Table 4). The most common native species

Table 3. Mean, standard error, minimum, and maximum values for surface water salinity, pH, temperature, nitrate-nitrogen (NO_3–N), ammonium-nitrogen (NH_4–N), and total dissolved nitrogen (TDN) across sites by island and across all islands for the sampling in March and April, 2007 ($n = 2$ samples per site).

	Salinity	pH	NO_3–N	NH_4–N	TDN
	‰		mg L^{-1}		
Kauai sites ($n = 8$)					
Mean	5.42	7.55	0.04	0.85	0.97
SE	2.43	0.24	0.02	0.74	0.50
Minimum	0.07	6.26	BDL†	BDL	0.06
Maximum	19.80	8.55	0.16	6.02	4.34
Oahu sites ($n = 11$)					
Mean	20.84	7.69	0.08	0.11	1.33
SE	6.28	2.32	0.02	0.03	0.40
Minimum	0.12	7.00	BDL	BDL	0.11
Maximum	138.26	8.57	0.77	0.77	4.87
Molokai sites ($n = 2$)					
Mean	18.60	9.09	0.02	0.02	2.64
SE	7.70	0.49	0.02	0.02	2.47
Minimum	10.71	8.60	BDL	BDL	0.17
Maximum	26.50	9.58	0.04	0.04	5.11
Maui sites ($n = 6$)					
Mean	12.59	8.43	0.17	0.04	1.70
SE	10.35	0.32	0.16	0.01	0.73
Minimum	0.15	7.75	BDL	0.01	0.04
Maximum	64.17	9.68	0.99	0.10	4.78
Hawaii sites ($n = 7$)					
Mean	8.05	8.09	0.14	0.03	0.20
SE	2.09	0.18	0.06	0.02	0.06
Minimum	0.14	7.25	BDL	BDL	0.06
Maximum	14.06	8.69	0.38	0.18	0.44
All sites ($n = 34$)					
Mean	12.99	7.95	0.10	0.25	1.16
SE	4.48	0.12	0.04	0.18	0.27
Minimum	0.07	6.26	BDL	BDL	0.04
Maximum	138.26	9.68	0.99	6.02	5.11

† BDL, below detection limit of instrument used for analyses.

sampled was water hyssop (*Bacopa monnieri* (L.) Wettst.), a codominant at 11 sites, followed by saltmarsh bulrush (*Bolboshoenus maritimus* (L.) Palla ssp. Paludosos), which was codominant at nine sites. The other two native species, Java sedge (*Cyperus javanicus* Houtt.) and sea purslane (*Sesuvium portulacastrum* (L.) L.), were codominant at six and two sites, respectively. The most common introduced species sampled was an aggressive-invasive California grass (*Urochloa mutica* (Forssk.) T.Q. Nguyen), which was codominant at eight sites (Table 4). Papyrus (*Cyperus papyrus* L.), sour grass (*Paspalum conjugatum* P.J. Bergius), and saltwater couch (*Paspalum vaginatum* S.W.) were each codominant at five sites. All other species were sampled at three or fewer sites. Site mean $\delta^{15}N$ values ranged from 1.1 to 14.7‰ (Table 2). Lower values (1.1– 4.9‰) were typically found in wetlands on the islands of Kauai, Hawaii, and Molokai, and higher values (8.7– 14.7‰) were found in wetlands on Oahu and Maui (Table 2).

Relationships of Plant Tissue $\delta^{15}N$ with Predictor Variables

The $\delta^{15}N$ values were positively correlated to percent development within a 1000-m radius ($r = 0.51$, $p < 0.001$) and human population densities ($r = 0.56$, $p < 0.001$) (Table 5). Site mean $\delta^{15}N$ values were also positively correlated to percent N content of plant tissue ($r = 0.37$) and TDN in surface water adjacent to the vegetation ($r = 0.37$) (Table 5). Although both of these correlations were relatively low, they were both significant ($p < 0.05$).

The $\delta^{15}N$ of the mixed plant community provided a better predictive model to identify N sources to Hawaiian wetlands compared with individual plant groups (i.e., grasses, sedges) or plant species. Linear regressions of $\delta^{15}N$ of mixed plant communities versus development within a 1000-m radius, forest cover within a 1000-m radius, and population densities pro-

Table 4. Mean plant tissue $\delta^{15}N$ and C:N by species (±1 standard error).

Species (*n*)†	Native versus introduced‡	Plant type	Mean $\delta^{15}N$	Mean C:N
Bacopa monnieri (11)	native	dicot herb	5.1 ± 1.4	26 ± 1
Cyperus javanicus (6)	native	sedge	5.5 ± 1.1	51 ± 4
Sesuvium portulacastrum (2)	native	dicot herb	6.9 ± 4.0	27 ± 7
Bolboschoenus maritimus (9)	native	sedge	6.9 ± 1.5	22 ± 1
Typha latifolia (1)	introduced	other monocot	1.1§	29§
Pennisetum setaceum (1)	introduced	grass	1.1§	65§
Cyperus involucratus (1)	introduced	sedge	2.0§	35§
Echinochloa crus-galli (1)	introduced	grass	2.9§	36§
Cyperus papyrus (5)	introduced	sedge	3.4 ± 0.8	37 ± 3
Paspalum vaginatum (5)	introduced	grass	3.5 ± 0.4	38 ± 3
Schoenoplectus californicus (2)	introduced	sedge	4.1 ± 1.9	31 ± 1
Ludwigia palustris (2)	introduced	dicot herb	4.3 ± 0.3	14 ± 1
Sagittaria latifolia (1)	introduced	other monocot	4.8§	9§
Urochloa mutica (8)	introduced	grass	5.1 ± 0.8	22 ± 1
Ageritum conyzoides (1)	introduced	dicot herb	5.3§	14§
Paspalum conjugatum (5)	introduced	grass	5.5 ± 1.6	24 ± 1
Ricinus communis (1)	introduced	dicot herb	6.3§	8§
Commelina diffusa (1)	introduced	other monocot	6.9§	14§
Panicum maximum (3)	introduced	grass	7.9 ± 3.9	25 ± 2
Eichhornia crassipes (1)	introduced	submerged herb	9.2§	12§
Chloris barbata (1)	introduced	grass	9.2§	21§

† This n value represents the number of sites where this plant species was present.

‡ According to Whistler (1994) and Erickson and Puttock (2006).

§ The standard error could not be calculated because only one sample was collected for this species.

vided similar, if not slightly better, fits ($r^2 = 0.27$, $p < 0.001$; $r^2 = 0.11$, $p < 0.05$; and $r^2 = 0.32$, $p < 0.001$, respectively) compared with grasses ($r^2 = 0.26$, $p < 0.001$; $r^2 = 0.10$, $p < 0.05$; and $r^2 = 0.29$, $p < 0.001$, respectively), sedges ($r^2 = 0.18$, $p < 0.05$; $r^2 = 0.15$, $p > 0.05$; and $r^2 = 0.14$, $p > 0.05$, respectively), and dicot herbs ($r^2 = 0.19$, $p > 0.05$; $r^2 = 0.11$, $p > 0.05$; and $r^2 = 0.22$, $p > 0.05$, respectively). Linear regressions could not be made using δ^{15}N from other monocots due to insufficient data for this plant group. Linear regressions for individual plant species often yielded r^2 values less than 0.2 that were not significant. Significant relationships were only observed between δ^{15}N values in tissue of *Bacopa monnieri* and percent development within a 1000-m radius ($r^2 = 0.32$, $p < 0.05$) and δ^{15}N values in tissue of *Urochloa mutica* and watershed population densities ($r^2 = 0.54$, $p < 0.05$).

The amount of developed (i.e., urban) land cover in a 1000-m radius of the site had a positive relationship ($p < 0.001$) with the individual plant δ^{15}N values (Fig. 2A). The δ^{15}N became enriched in ^{15}N by 0.1‰ for each percent increase in developed area within the radius. In contrast, the amount of forested land use in the 1000-m radius had a negative relationship ($p = 0.05$) with individual plant δ^{15}N values (Fig. 2B). The δ^{15}N became depleted in ^{15}N by 0.1‰ for each percent increase in forested area. There was also a positive relationship ($p < 0.001$) with individual plant δ^{15}N values and watershed population density, with the more densely populated watersheds having δ^{15}N values more enriched in ^{15}N (Fig. 3).

Discussion

Previous studies that have examined relationships between δ^{15}N of wetland plants and watershed land use have largely focused on comparisons of single plant species across different sites. For example, the δ^{15}N values of *Spartina alterniflora* were positively correlated to human N inputs in New England salt marshes (McClelland and Valiela, 1998; Valiela and Cole, 2002; Cole et al., 2004). Similar correlations have been reported for *Iva frutescens*, *S. patens*, and *Phragmites australis* (Wigand et al., 2007). However, in Hawaii, plant community structure differs considerably among wetlands (Bantilan-Smith, 2008). This is largely a result of the isolated nature of Hawaii's wetlands and the introduction of exotic plants. Thus, intersite comparisons of the same plant species can be difficult if not impossible because one species does not occur across all coastal wetland sites. However, in this study we have provided evidence that the δ^{15}N of mixed plant communities, compared with the δ^{15}N of individual plant groups (i.e., grasses, sedges) or individual plant species, shows promise as a potential indicator of N sources to these wetlands.

Watershed Land Use, Demographics, and Water Quality Data

The considerable differences in δ^{15}N values among sites were attributed to the wide range of adjacent land uses, population densities, and potential differences in N sources in their watersheds. Because N isotopic ratios have been used to estimate the extent to which anthropogenic N inputs have been incorporated into the biota at specific sites in North America (Pruell et al., 2006), this technique also appears to be valid for Hawaii especially given the wide range in δ^{15}N values across the 34 sites (1.1–14.7‰).

Table 5. Pearson's correlations of δ^{15}N values of herbaceous wetland plants with the environmental, watershed, and demographic variables measured in this study.

Variable	Correlation with δ^{15}N
Plant tissue total N, %	*0.37*†
Surface water temperature, °C	0.09
Surface water dissolved oxygen, mg L^{-1}	0.13
Surface water pH	–0.01
Surface water conductivity, ms cm^{-1}	0.29
Surface water salinity, ‰	0.30
NO_3–N, mg L^{-1}	0.30
NH_4–N, mg L^{-1}	–0.10
Total dissolved nitrogen, mg L^{-1}	*0.37*
PO_4–P, mg L^{-1}	0.20
Total phosphorus, mg L^{-1}	0.06
Development within a 1000-m radius, %	*0.51*
Agriculture within a 1000-m radius, %	–0.23
Grass/scrubland within a 1000-m radius, %	–0.08
Forest within a 1000-m radius, %	–0.33
Wetland within a 1000-m radius, %	0.33
Shoreline within a 1000-m radius, %	–0.19
Bare ground within a 1000-m radius, %	–0.24
Open water within a 1000-m radius, %	0.03
Population density, people ha^{-1}	*0.56*

† Italic values denote significant correlations ($p < 0.05$).

The extremely high mean salinity value (138.26‰) at the Pouhala site on Oahu was most likely due to high evaporation rates and poor hydrologic flushing, which leads to a build-up of ions in the water column. The high mean NO_3–N values observed for sites on Maui most likely relate to the agricultural (sugar and pineapple operations) land-use activities in these watersheds upslope of the wetlands. The high NH_4–N value observed at the Kauaihau Riparian site was probably related to N inputs from cattle that were actively grazing this site at the time of sampling. Total dissolved N values were high for sites on Oahu, Molokai, and Maui, suggesting that organic N rather than inorganic N may be the dominant form of N in surface waters of these sites.

Plant Tissue δ^{15}N among Species and Sites

The higher δ^{15}N values from Klipper Pond and Waiawa were attributed to inputs of sewage because the δ^{15}N values in the plant tissue at these two sites were similar to δ^{15}N observed in waste water (10.1‰; Derse et al., 2007) and because both sites are located in densely populated urban areas and are near sewage treatment plants. Four other wetlands on Oahu and three wetlands on Maui had high plant δ^{15}N values (>8‰), suggesting N enrichment from sewage. The five Oahu wetlands with the highest δ^{15}N values (Klipper Pond, Waiawa, Hamakua, Pouhala, and Kawainui) were located in urban watersheds that supported populations of >10 people ha^{-1}, were >50% developed within a 1000-m radius of the sites, or both (Table 2).

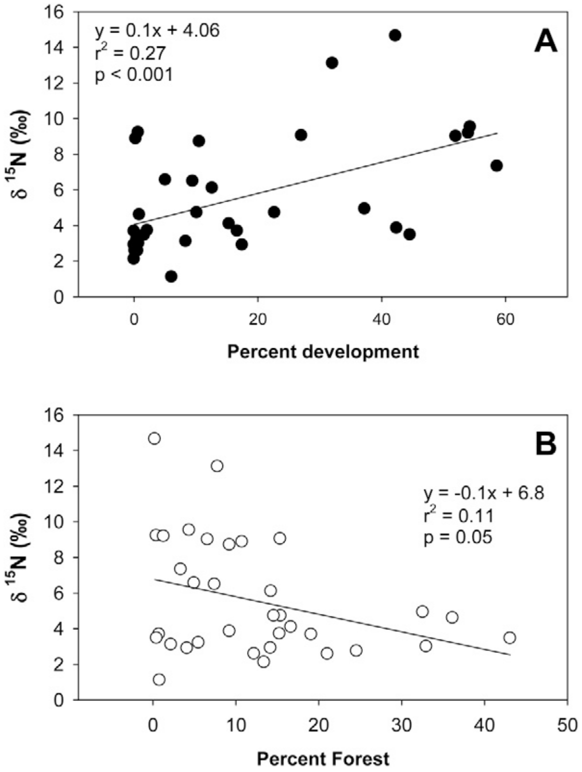

Fig. 2. Relationship between site mean δ^{15}N values in plant tissue and (A) percent development within a 1000-m radius of the wetland sites and (B) percent forest within a 1000-m radius of the wetland sites. Site mean δ^{15}N values were positively correlated to percent development ($p < 0.001$) and negatively correlated to percent forest ($p < 0.05$).

The Maui wetlands sampled were generally located in watersheds with lower levels of development within a 1000-m radius (0.1–4.9%) and lower population densities (0.08–3.2 people ha^{-1}) compared with Oahu. However, large areas of land within a 1000-m radius of the Pakukalo and Waihee (23.8%), Kealia (38.1%), and Kanaha (43.5%) sites were classified as agricultural. Furthermore, treated sewage water is disposed of in various areas of Maui by injecting it into saltwater underlying the fresh ground water lens. In other watersheds on the western coast of Maui, nutrient inputs from agriculture and sewage injection wells have been linked to nuisance algal blooms that occur in nearshore waters (Soicher and Peterson, 1997). The high δ^{15}N values of macroalgae responsible for these blooms (7–8‰) support the idea that injection wells are the cause of these nearshore blooms (Smith et al., 2005). The macroalgal δ^{15}N values in the Smith et al. (2005) study were similar to the δ^{15}N values we observed in the wetland plant tissues of Maui wetlands. Thus, it is likely that the elevated δ^{15}N values from Pakukalo and Kealia were a result of sewage injection wells. Another study of coastal waters in South Florida found high tissue δ^{15}N values (+10 to +12‰) of fleshy macroalgae (*Codium isthmodcladum*) that the authors concluded were a direct result of nutrients from leaky septic tanks, sewage outfalls, and injection wells (Lapointe and Clark, 1992). The high δ^{15}N values reported from the other Maui wetland, Nuu, were likely due to a cattle egret (*Bubulcus ibis*) rookery that is located at the periphery of the wetland. Each year, more than 200 egrets roost in this wetland (S. Fisher, Maui Coastal Land Trust, personal communication) and likely contribute a significant amount of N to this wetland.

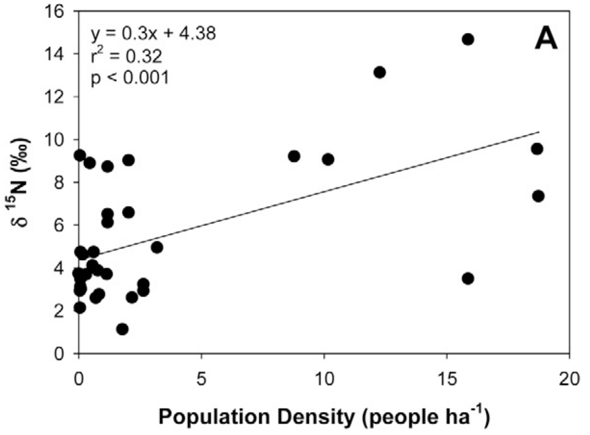

Fig. 3. Relationship between site mean δ^{15}N values in plant tissues and watershed human population densities.

Alternative explanations for the high δ^{15}N values from the Oahu and Maui wetlands could be processes occurring within the wetlands that result in enriched δ^{15}N values of DIN pools. These processes include nitrification, ammonia volatilization, denitrification, and plant or algal uptake. All of these processes use the lighter ^{14}N isotope at a faster rate than the ^{15}N isotope, leaving the remaining DIN pool and the plants or algae that use them isotopically enriched with ^{15}N (Cifuentes et al., 1989; Anderson and Fourqurean, 2003; Fry et al., 2003). Previous research has shown that the fractionation factors involved in various transformations of N vary among ecosystems and specific organisms (Fry et al., 2003; Lamb and Swart, 2008). Thus, normal ecosystem processes, such as nitrification and denitrification, which occur regularly in coastal wetlands, can result in δ^{15}N values that are misinterpreted as evidence of anthropogenic influence (Lamb and Swart, 2008). Therefore, further research is needed to determine the relative contributions of atmospheric inputs, diffusion from sediments, agriculture, sewage, egret rookeries, nitrification, denitrification, and assimilation to the δ^{15}N values of plant tissues in these coastal Hawaiian wetlands.

Most of the 21 wetlands with the lowest δ^{15}N values (1.1–4.9‰) were on the islands of Hawaii, Kauai, and Molokai. The land within a 1000-m radius of the wetlands on these islands is less developed (<10%) and supports smaller human populations (<3 people ha^{-1}) compared with Oahu or Maui. Two of these 21 sites were located on Oahu. The first site, Waimea, had <1% developed land use within 1000-m of the site and a population of <1 people ha^{-1}. The second site, Percolation Ditch, also had low δ^{15}N values. This wetland is located near residential lawns and a golf course that receive commercial fertilizers, which may account for the lower δ^{15}N values of wetland plants (McClelland et al., 1997; Wigand et al., 2007). The lower δ^{15}N values of wetland plants from the other 20 sites could also be explained in part by inputs of commercial fertilizers or less contribution from human waste water at these sites (McClelland and Valiela, 1998).

Alternatively, the low $\delta^{15}N$ values reported from these 21 wetlands could be due to the fact that N is not limited in these systems. When N is limited, plants have $\delta^{15}N$ values similar to their N source. As more N becomes available, disproportionate use of ^{14}N over ^{15}N leads to $\delta^{15}N$ values that are lower than their N source (Wada and Hattori, 1978; Pennock et al., 1996; McClelland and Valiela, 1998). However, the average N:P ratio from plants in these wetlands was 10.0 ± 0.8 (range, 2.9–17.5), which suggests that N is a limiting nutrient in these wetland ecosystems (Verhoeven et al., 1996) and that the $\delta^{15}N$ of the plants sampled represents their N source. Furthermore, NO_3–N and NH_4–N concentrations in the water column adjacent to vegetated areas were often at or below the detection limit for these sites (Table 3).

The introduction and establishment of introduced species across coastal lowland wetlands in Hawaii may also have influenced the site mean $\delta^{15}N$ values. A concurrent study of vegetative species composition and cover at these sites reported that only 16 of the 85 plant species identified were native. Three of the four most abundant species were considered highly invasive (Bantilan-Smith, 2008). Because many of these introduced species have different N acquisition, translocation, and storage rates, can access N more efficiently, or are capable of thriving at higher levels of N, we expect that there has been and will continue to be a shift in the $\delta^{15}N$ values in the plant tissues across these sites over time. More research and more comprehensive sampling of a wider range of species, sites, and seasonal variability are needed to draw further conclusions on this topic. The weak relationships we reported when $\delta^{15}N$ values of individual plants were examined may be strengthened by sampling a larger set of sites with similar plant communities (i.e., one or two common species at all sites). This may be a useful approach to evaluate changes in $\delta^{15}N$ values while holding species constant and varying land-use or N sources. However, this may be a difficult task in Hawaii because plant communities significantly vary within and across the five main islands (Bantilan-Smith, 2008).

Relationships of Plant Tissue $\delta^{15}N$ with Predictor Variables

The influence of humans on the $\delta^{15}N$ values of wetland plants was further demonstrated by the significant and positive correlations of $\delta^{15}N$ values with percent development within the 1000-m radius of the sites and watershed population densities. These two variables were better predictors of $\delta^{15}N$ values than were plant tissue N content or any of the water quality parameters. Higher $\delta^{15}N$ values were attributed to increased sewage inputs. Such inputs likely include cesspools, septic tanks, leaks in sewage lines, and animal wastes that are associated with higher human population densities and urbanization. Increased sewage-derived N would result in an increase in the amount of heavier ^{15}N isotope available for wetland plant uptake and an increase in $\delta^{15}N$ values of wetland plants (McClelland and Valiela, 1998; Cole et al., 2004). The decrease in $\delta^{15}N$ values with the increase in forested area within the 1000-m radius of the wetland sites was most likely due to lower N inputs from the forested ecosystems, which generally do not export as much N as agricultural or urban land uses.

Similar correlations between $\delta^{15}N$ values in aquatic ecosystems and human activities have been reported elsewhere. A comparison of $\delta^{15}N$ of various consumers (i.e., herbivorous fish, mussels, chironomids, isopods, etc.) from 11 different studies revealed that $\delta^{15}N$ values increased significantly with human population densities in surrounding watersheds. Increased $\delta^{15}N$ values were attributed to increased ^{15}N inputs from human sewage (Cabana and Rasmussen, 1996). Significant relationships were also reported when $\delta^{15}N$ values from fish (i.e., largemouth bass [*Micropterus salmoides*] and yellow perch [*Perca flavescens*]) and unionid mussels (*Elliptio complanata*) and the fraction of residential development in 91.4-m buffer zones were compared across 17 small lakes in Rhode Island (Lake et al., 2001). Similar patterns have been reported from coastal ecosystems. For example, Wigand et al. (2001, 2003) reported positive correlations between $\delta^{15}N$ in *S. alterniflora* in Narragansett Bay salt marshes and percent residential land use in coastal watersheds in Rhode Island. Increased inputs of waste water, which are positively correlated to urbanization of coastal areas, have also resulted in higher $\delta^{15}N$ values of macroalgae, macrophytes, and consumers (Hansson et al., 1997; McClelland and Valiela, 1998; Cole et al., 2004).

The results from our study and others mentioned herein suggest that increasing human population densities in coastal watersheds lead to increasing N inputs to coastal waters, which have the potential to overwhelm the baseline nutrient processing and removal capacities of these systems. Valiela and Cole (2002) identified a threshold of 20 to 100 kg N ha^{-1} yr^{-1} for N inputs to coastal wetlands. Once N inputs exceed these levels, the ability of coastal wetlands to remove excess N is overwhelmed, and the elevated anthropogenic N loading increases the amount of N leaking from coastal wetlands to nearshore ecosystems. Thus, even though the increase in percent N content of plants and $\delta^{15}N$ suggests that plants in Hawaiian wetlands have the potential to sequester increased N loads, the efficiency with which these ecosystems remove increased N loads may be compromised as human populations increase. These results also suggest that new sewage treatment plants and updates to existing sewage infrastructure may be needed in developing and developed watersheds in the state of Hawaii as well as throughout the western Pacific region to protect coastal ecosystems because sewage treatment systems have been shown to be major sources of N to coastal systems in other areas (Nixon et al., 1995).

Future monitoring of $\delta^{15}N$ in Hawaii wetlands should also consider sampling design. Cross-site comparisons of $\delta^{15}N$ values appear to be an effective technique for tracking N inputs to coastal ecosystems in Hawaii and elsewhere (Wigand et al., 2001, 2003). However, the low r^2 values reported here suggest that the usefulness of these comparisons is limited, especially when $\delta^{15}N$ values are low. Intensive collection of samples from within a single wetland may prove more effective in identifying N sources. Not only can this sampling design determine the spatial extent that anthropogenic inputs influence coastal ecosystems (Costanzo et al., 1999; Wayland and Hobson, 2001; Savage, 2005), but it can also help identify N sources (Brion et al., 2000; Fry et al., 2003). Fry et al. (2003) developed a model that used DIN concentrations and δ^{15}N values throughout an estuary to identify sources of enriched δ^{15}N inputs. Unfortunately, this sampling design would also result in the loss of spatial resolution across the Hawaiian Islands because fewer wetlands could be sampled. Perhaps a better approach would be to sample a subset of wetlands over a longer time period. Algal samples collected over time from Australian estuaries were effective at tracking decreased inputs of anthropogenic N (Costanzo et al., 1999).

Conclusions

The significant differences in δ^{15}N values across the 34 wetland sites were attributed to differences in surrounding land use and watershed population size. Specifically, increased cover of developed land and human population densities were positively correlated with δ^{15}N values in coastal wetland plant tissues. Increased δ^{15}N values were attributed to increased sewage inputs, which are likely due to cesspools, septic tanks, leaks in sewage lines, and animal wastes as such N sources are higher in ^{15}N compared with other inputs, such as artificial fertilizers or N fixation. Our results suggest that sewage appears to be a significant source of N to coastal wetlands on Maui and Oahu. Although more research and a more comprehensive sampling of a wider range of species, sites, and seasonal variability are needed to conclusively show the utility of δ^{15}N for tracking N sources, the use of δ^{15}N values from multiple plants across different wetland ecosystems shows promise. Furthermore, the results from this study can be used as a baseline data set to monitor future changes in N inputs to these coastal wetlands, especially with increases in human populations predicted to occur in Hawaii and in other Pacific Islands over the next few decades.

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