AN EXAMINATION OF HOW HUMAN AND LARGE WILDLIFE POPULATIONS AFFECT THE NITROGEN BUDGET OF THE MARA RIVER BASIN, EAST AFRICA

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Abstract. Nitrogen is a key nutrient in ecosystems worldwide, but also has the capacity to be a potent pollutant. We developed a nitrogen budget for the Mara River Basin in East Africa and examined the effect of wildlife on inputs, flux, and retention rate in the river. We estimated nitrogen inputs to the watershed using the net anthropogenic nitrogen input (NANI) method, and estimated N flux both using the USGS load estimation program LOADEST and as an average of concentration and discharge data. We applied these methods to two distinct portions in the watershed—an upstream area (2,454 km^2, bounded by site 1), with major human inputs and low wildlife populations, and a downstream area (6497 km^2, bounded by site 2), that is larger but with proportionately less human influence and more wildlife. Nitrogen inputs for both sites came primarily from human and livestock consumption (47-53%). Atmospheric deposition and biological fixation of N both contribute an average of 39.5% and 9%, respectively, of the total basin N, while fertilizer application and wildebeest carcass deposition both account for <1%. Riverine N flux accounts for 0.3-14.9% of the total inputs at both site 1 and site 3, meaning 85.1-99.7% of the total N inputs were retained in the river. This retention rate is higher than many other watersheds globally. Wildlife has a minimal effect on N retention rate in the Mara River, suggesting humans are the main drivers of N loading and flux. Careful management of human and livestock waste must be put in place to control pollution of the Mara River. Cropland management strategies may also be necessary to prevent excess soil N depletion and further environmental and economic problems.

INTRODUCTION

Nitrogen is often a major determinant of primary productivity, and is a limiting nutrient in many terrestrial and marine ecosystems (Vitousek et al., 1997). Approximately 78% of the atmosphere is composed of diatomic nitrogen (N_2), which is unavailable to most organisms due to the strength of bond between the two atoms (Galloway et al., 2004). Over the course of evolutionary history, a select few Bacteria and Archaea have developed the means to fix atmospheric nitrogen into biologically available forms, also referred to as reactive nitrogen (Nr). This fixation can occur in both free-living bacteria and those in symbiotic relationships with leguminous plants. Nr exists in two major forms in terrestrial and aquatic ecosystems, reduced (NH_4^+) and oxidized (NO_x). Different species of bacteria have the mechanisms to convert between these two major forms, as well as convert NO_x back into N_2 through the process of denitrification.

Soil nitrogen is often highly mobile, which in turn leads to a high connectivity between terrestrial and aquatic ecosystems. After fixation, nitrogen is readily lost from terrestrial ecosystems by leaching and runoff to aquatic systems, and through denitrification and volatilization of organic N to the atmosphere (Vitousek and Howarth, 1991). These losses often lead terrestrial ecosystems to be N limited, and studies have shown that fertilizing with nitrogen alone can increase primary productivity in a wide variety of habitat types (Breman and Dewit, 1983; Hunt et al., 1988). In contrast, primary production in most freshwater ecosystems is limited by phosphorus, not nitrogen. However, many phytoplankton species in

these aquatic ecosystems can also be limited by nitrogen when the availability of nitrogen is low compared to that of phosphorus (Vitousek and Howarth, 1991).

Human activities have greatly altered the cycling of N through both terrestrial and aquatic ecosystems through two major avenues: food and energy production (Galloway et al., 2004). In food production, leguminous crops increase terrestrial N stocks through N_2 fixation via their symbiotic bacteria. However, many crops lack symbiotic bacteria, and because terrestrial ecosystems are often N limited, supplemental Nr is required to sustain crop growth year to year. This need led to the development of the Haber-Bosch process in the early 1900s, an industrial process used to produce NH_3 from N_2 and H_2 (Galloway et al., 2004; Howarth et al., 1996). Energy production, primarily the burning of fossil fuels, adds N to the atmosphere as a byproduct (NO_x) from either the oxidation of atmospheric N or organic N in the fuel (Galloway et al., 2002). Nr in the atmosphere can then be deposited on the landscape, potentially hundreds of miles from its source.

Despite N being limiting in many ecosystems, excessive N can induce a variety of environmental problems (Vitousek et al., 1997). Not only are NO_x potent greenhouse gases, but they also contribute significantly to acid rain. Increases of Nr in soils can lead to leaching of other essential nutrients such as calcium and potassium, as well as overall acidification of the soils. This highly mobile (labile) soil N can quickly enter streams and rivers, leading to acidification and dramatically increased loading in estuaries and coastal areas. Abundant N in these formerly limited aquatic areas can lead to rapid algal growth and die off, creating anoxic zones and greatly disturbing the local ecosystem, a process referred to as eutrophication.

Loading of nitrogen in ecosystems is highly influenced by human activity and by animals, which can both import nitrogen from other ecosystems and increase rates of nitrogen cycling within an ecosystem (Howarth et al., 1996; Masese et al., 2015; Quynh et al., 2005; Seitzinger et al., 2002; Subalusky et al., 2015; Ti et al., 2012; Zhou et al., 2014). In order to study this cycle, researchers can create a nutrient budget that quantifies the amount of nutrients entering, leaving, and remaining in a given area. The most common and convenient area of study in which to create nutrient budgets is a watershed, which is the area of land that drains into a single river, and in the context of this paper I will use watershed, catchment, and (river) basin interchangeably. Common variables analyzed in these studies include how much N is added to the river basin both by humans and animals (input), how much N the river carries out of the basin (flux), and how much N stays within the basin (retention rate). Retention rate can be further broken down into components including denitrification, biological uptake, and sediment sorption. Biological uptake is simply the amount of nitrogen taken up by organisms, and sediment sorption is the amount that adheres to sediments on the riverbed. Extensive N budgeting has been done in the northern hemisphere for river basins in Southeast Asia (Quynh et al., 2005; Ti et al., 2012), the North Atlantic watersheds (Howarth et al., 1996), and the northeastern United States (Boyer et al., 2002; Groffman et al., 2004; Seitzinger et al., 2002).

Human contributions of N to ecosystems are compressed into one term and referred to as net anthropogenic nitrogen inputs (NANI) (Howarth et al., 1996). These inputs include fertilizer application, crop fixation of nitrogen, atmospheric deposition, and net import of agricultural products. Net import of N is evaluated as the difference between nitrogen added by livestock and human waste and nitrogen removed through crop and livestock production (Hong et al., 2011). In addition, some studies have examined biofuel burning, including crop residue and fuel wood, as a pathway from terrestrial to atmospheric nitrogen. Fertilizer application is by far the leading contributor of nitrogen to ecosystems in developed countries, particularly after the development of the Haber-Bosch process. However, differing access to nitrogenous fertilizers and industrial fixation makes global fertilizer inputs quite variable (Potter et al., 2010).

Many studies on nitrogen budgets have focused exclusively on anthropogenic effects, and the role of animals in altering nutrient cycling within an ecosystem has been largely underappreciated. Animals can act as important vectors for nutrient transfer between and within ecosystems, and are particularly important because they allow for nutrient movement against abiotic gradients (Kitchell et al., 1979; Vanni, 2002). For example, Pacific salmon gain roughly 95% of their body mass from marine environments, and during their reproduction migration they bring these nutrients up the naturally established gradient and deposit them in lakes, streams, and riparian areas upon death (Naiman et al., 2002). Additionally, these nutrients have been found to be critically important to the productivity in these aquatic ecosystems. The two primary ways in which animals transfer nutrients between ecosystems are carcasses, like the salmon, and nutrient elimination (excretion and egestion, or liquid and solid waste, respectively) (Kitchell et al., 1979; Vanni, 2002). Deposition of carcasses is the better-known method of transfer and most often occurs as a pulse input during long distance feeding or reproductive migrations, natural disasters, and sudden shifts in environmental regime (Weigelt, 1989). In contrast to carcass deposition, nutrient elimination inputs often occur in lower quantities, but are relatively consistent throughout the year. Excretion is often labile inorganic nitrogen that is readily transported and absorbed by organisms, whereas egestion is organic and must be mineralized before use (Vanni, 2002). Despite abundant knowledge on how animals affect nutrients in ecosystems, little research has been done to discover how wildlife inputs compare to and interact with anthropogenic inputs.

There are many different factors affecting N inputs to river basins, but discharge plays the biggest role in determining the amount of nitrogen coming out of the system. Nitrogen flux has the potential to be much higher during high flow events where the water has enough energy to mobilize organic matter that had previously settled out of the water column during low discharge periods. Lower discharge rates allow for a greater time of sediment-water contact, which promotes retention processes such as denitrification and vegetative uptake. The result is that differences in discharge almost entirely explain differences in nitrogen retention rates in different bodies of water (lakes, rivers, wetlands) (Saunders and Kalff, 2001).

In East Africa, animal nutrient subsidies are incredibly important because large wildlife populations still exist here, some of which undertake annual migrations, and the scale at which nutrients are transported is vastly different than many other places in the world. For example, the Mara River Basin in Kenya and Tanzania sustains a resident population of over 4000 hippos, and this population is a major contributor of nitrogen via nutrient elimination (Kanga et al., 2011). Hippos graze from nearby grasslands during the night and defecate much of it into the river during the day, and this constant large-scale shuttling of nutrients and organic matter from terrestrial to aquatic systems is what makes hippos different from most other large herbivores (Subalusky et al., 2015). The Mara also hosts the Serengeti wildebeest migration, in which 1.3 million wildebeest migrate north into the Mara River basin during the dry season each year. During this migration, wildebeest cross the Mara River multiple times and periodic mass drownings can occur, providing pulse nitrogen inputs via carcasses (Subalusky, 2016). Although research has shown that hippos and wildebeest are important vectors for carbon, little has been done to quantify their effect as broader nutrient vectors (Masese et al., 2015; Subalusky et al., 2015).

Ungulate grazers affect nitrogen cycling and retention in a variety of ways, and evidence has been found showing that they can both increase and decrease rates of retention within a single ecosystem. Frank et al. (2000) found that ungulate grazing increased nitrogen retention in soils by stimulating microbial activity, likely by increasing labile soil carbon. Wildebeest bring in nutrients from distant areas like Serengeti National Park and other southern plains on their migration route, which greatly enhances primary productivity in grasses in the weeks following their passage (McNaughton, 1976). However, in areas heavily trampled by wildebeest and other migratory animals, new grass growth is likely severely diminished, which could increase runoff of soil and the nitrogen it contains. In the Amazon River Basin, it was found that nitrogen-rich litterfall reduced overall nitrogen retention by mobilizing terrestrial N into the river directly, effectively speeding up the transport process (Howarth et al., 1996). By extension, it is

possible that hippos have a similar effect, grazing on land overnight and then returning to the river and defecating and urinating much of that nitrogen into the river.

Understanding nitrogen cycling in this part of the world is of critical importance because much of the East African economy is dependent upon small-scale agriculture and tourism. As of 2014, 68.5% and 73.8% of the labor forces in Kenya and Tanzania, respectively, depended on agriculture for their livelihood (Food and Agriculture Organization of the United Nations, 2015). Many areas in the Lake Victoria Basin lack adequate reactive nitrogen inputs to compensate for crop removal, which may lead to mining of soil organic nitrogen and greatly diminished soil fertility if not addressed (Davidson, 2009; Zhou et al., 2014). Additionally, tourism in these countries is incredibly dependent on the national parks and game reserves, which in 2014 accounted for 10.5% and 13.3% of the GDP in Kenya and Tanzania, respectively (World Travel and Tourism Council, 2015). Understanding how nitrogen behaves in this basin is critical because it will allow people to plan for potential future environmental and economic problems.

Our goal in this project was to create a nitrogen budget for the Mara River Basin and calculate flux out of the system at Emarti (site 1), which is upstream of wildlife inputs, and at New Mara Bridge (NMB, site 2), which is downstream of both hippo and wildebeest inputs (Figure 1). Both sites are downstream of human populations. I explored these fluxes by quantifying both anthropogenic and wildlife inputs to the system, and by estimating the retention rate in the basin and how it might be affected by the different inputs. I expect large wildlife, such as hippos and wildebeest, to alter local nitrogen cycling by mobilizing terrestrial nitrogen stocks directly into the river. If this is the case, I hypothesize that 1) nitrogen flux will be higher at the NMB, and 2) retention rate will be lower at the NMB, both due to the presence of large mammals.

METHODS

Study Area

The Mara River flows through Maasai Mara National Reserve (MMNR) and Serengeti National Park (SNP) before entering Lake Victoria (Fig. 1). The average discharge in the Mara is 16.8 m^3 s^{-1} over the historical record, but can range from <1 to >80 m^3 s^{-1} depending on rainfall patterns (LVBC and WWF-ESARPO, 2010). Most of the human population exists in the upper catchment of the river (upstream of Emarti/Site 1), allowing for distinction between human and natural inputs by sampling at a site just upstream of the beginning of significant wildlife populations. Sampling at site 2 incorporates the influence of large wildlife. Because there are distinct areas of human and wildlife influence in the catchment, it is possible to differentiate human and wildlife effects on nitrogen loading, flux, and retention rate in the Mara basin. It is important to note that, unlike many other analyses comparing watershed nitrogen budgets and retention rates, site 1 is nested within site 2 and will thus have a downstream effect. The two most important large mammal nutrient vectors in this ecosystem are hippopotamus (*Hippopotamus amphibius*) and wildebeest (*Connochaetes taurinus*).

Net Anthropogenic N Input

The NANI model is the sum of atmospheric deposition, N fertilizer application, crop N_2 fixation, and net import of food and feed. We estimated anthropogenic inputs based on the calculations described in Howarth et al. (1996) and wildlife inputs based on loading estimates that have been developed for hippo and wildebeest inputs in the Mara (Subalusky, 2016; Subalusky et al., 2015).

Atmospheric N deposition includes wet and dry deposition of both oxidized and reduced nitrogen. Basin-wide deposition was estimated using measurements taken at three stations through the International

Global Atmospheric Chemistry Debits Africa (IDAF) network in dry savanna ecosystems (Delon et al., 2010).

Fertilizer application estimates were done using the Global Fertilizer and Manure (v1) database (Potter et al., 2010). The data were compiled through the Columbia University Socioeconomic Data and Applications Center and are presented at a 0.5° resolution. Total cropland area was derived as a percentage of the total land area (Ramankutty et al., 2008); the gridded data are provided at a resolution of 0.083°. Because of the coarseness of the fertilizer gridded data, the basins for the two sites only overlapped several data blocks and occupied these blocks to varying degrees. We did not estimate the proportion of each block within the basin, therefore an un-weighted average of fertilizer application rate was calculated for each basin. The average was then multiplied by cropland area to determine total annual fertilizer application.

Agricultural N fixation was estimated by multiplying local crop area by the global average fixation rate per area for each crop type. Major legumes grown in the Mara River Basin are cowpeas and beans. Crop areas were derived from the world census database 'Harvested Area and Yield of 175 Crops' (M3-Crop Data) (Monfreda et al., 2008). The gridded data are provided at a resolution of 0.083°. The average annual fixation rates are 90 kg N ha^{-1} for beans and 19.5 kg N ha^{-1} for cowpeas (Naab et al., 2009; Smil, 1999; Zhou et al., 2014).

NANI estimates also include net import of food and feed, which is considered as the difference between 1) human and livestock consumption and 2) livestock and crop production. Annual human consumption was calculated by multiplying the per capita N consumption by the total population. Human population for the basin is provided in Hoffman (2007), with the Bomet and Nakuru districts feeding into site 1, and Transmara, Narok, Bomet, and Nakuru districts feeding into site 2. Annual consumption of nitrogen per person was calculated based on daily protein intake and protein nitrogen content (Schoenfeldt and Hall, 2012).

Livestock included the five most prevalent animals in the basin: cattle, sheep, goats, pigs, and poultry (chickens). Sheep and goats were found to be identical in their nitrogen consumption and production and will be referred to as 'shoats' hereafter. Livestock consumption was calculated by multiplying livestock population by average annual N consumption per animal (Delve et al., 2001; Hong et al., 2011; Machila et al., 2008). Production was estimated using the N content in the edible portions of these animals, including beef, pork, lamb, chicken meat, and eggs (Hong et al., 2011). Basin wide estimates were calculated by multiplying edible N per animal by population. Livestock populations were derived from the 'Gridded Livestock of the World', which was presented as population density at a resolution of 0.05° (Robinson et al., 2014).

Major crops in the basin include maize, wheat, sorghum, sweet potatoes, millet, beans, cassava, bananas, and tea, and crop production was estimated by multiplying annual yields derived from 'Harvested area and yields of 175 crops' by the corresponding nitrogen content in each crop (Hong et al., 2011; Lander et al., 1998; Monfreda et al., 2008; Parikh et al., 1994; Sitienei et al., 2013; Yeoh and Truong, 1996).

In addition to average anthropogenic inputs, we also calculated high and low NANI estimates using the same sources and methods outlined in this section. The analysis of all spatial data was done with the use of ArcGIS 10.1.

Wildlife Inputs

Wildebeest were considered an input for site 2 (NMB) in the form of carcasses deposited in the river during migration because a large proportion of their biomass is accumulated in the southern Serengeti

during the wet season, which is outside the boundary of the Mara River basin. We multiplied nitrogen content per carcass by the average number of carcasses per year to determine yearly wildlife loading (Subalusky, 2016). We further refined our estimate by determining the average number of days per year wildebeest spend in the Mara (172 days) and scaled nitrogen loading down proportionately to remove body mass gained in the basin from the input estimation.

Although hippos are also a major source of nitrogen for the Mara River and may influence nutrient flux and retention rates, we did not consider them as inputs in this study (Subalusky et al., 2015). During their daily feeding migration, they travel 1-10 km inland to forage, almost always keeping them well within the bounds of the basin. Similar to hippo nutrient elimination, there are many other inputs to the river coming from within the basin, but this study is focused on inputs to the watershed as a whole, which necessitates the exclusion of hippo inputs to the river from our analysis.

Flux Data Collection

Discharge data were collected at both the Emarti and New Mara Bridges. At the Emarti site, stage height (river height at a stationary gauge, used as a proxy for discharge) was measured every 15 minutes from June 2011 – December 2014 using a WinSitu depth transducer and corrected with a WinSitu barometric pressure logger (In-Situ Inc., Fort Collins, CO, USA). At New Mara Bridge, stage height was measured every 15 minutes from Jun 2011 – Nov 2012 using a WinSitu depth transducer and barometric pressure logger, and from Dec 2012 – Dec 2014 using a depth transducer probe connected to a Eureka Manta2 sonde (probe used to measure stage height; Eureka, Austin, TX, USA). Rating curves were developed for both sites by measuring discharge on multiple dates in 2011 and 2014 using the area-velocity method. In the rating curve, precisely measured values of discharge were plotted against stage height, and an exponential curve was plotted from which all other stage height values were calculated.

Water samples were gathered to measure nutrient content from 2011-2014 in roughly 3-month periods (n = 16 and 36 for sites 1 and 2, respectively), and were kept cold (<4°C) after collection and processed as soon as possible. To measure total nitrogen (TN), unfiltered samples were acidified for preservation, diluted to acceptable sediment levels (<150mg/L) to avoid interference with colorimetric measurements, digested using an alkaline potassium persulfate digestion reagent, and analyzed on an Astoria-Pacific flow analyzer.

Estimation of Flux

Discharge and TN measurements were taken at different intervals over the course of the study, and this led to some gaps in our calibration data. Because of the limited number of nutrient measurements (n = 16 and 36 for sites 1 and 2, respectively) we extrapolated discharge values for the few concentration measurements that were lacking discharge data. Where possible, missing discharge measurements were calculated as a daily average and the time marked as noon (1200). The missing discharge measurement for site 1 on 25 February 2014 was estimated from discharge for that date at site 2 by creating a linear regression comparing base flows at sites 1 and 2 from 1-15 February 2014 (without the influence of a flood pulse on 8-9 February). In addition to base flow samples of TN, flood pulse TN measurements were taken at site 2. From the 4 pulses measured, the highest TN measurement was used in conjunction with discharge at the same time in model calibration. The highest value was chosen to provide the calibration with flood pulse data, and only one point was used so as not to bias the model toward flood pulse constituent loads.

Nitrogen flux at sites 1 and 2 was calculated using regression modeling in the USGS load estimation program LOADEST (Runkel et al. 2004). LOADEST estimates constituent loads (N concentration) in streams using time-series streamflow (discharge) and constituent concentration data to calibrate a

regression model. Variables included time-linked discharge and total nitrogen and we had the program select the 'best' of 9 premade models based on AIC (Akaike Information Criteria). The output included estimated daily load for the specified time period, standard error, and 95% confidence intervals. We ran this calibrated model using two distinct discharge data sets from each site to determine average annual flux of N. First, using the time linked calibration discharge data (site 1 n=16, site 2 n=36) and second, using all time linked discharge data collected for the period of study (site 1 n=982, site 2 n=902). All flux data were analyzed with the rLoadest package using R software (Lorenz et al., 2015; R Core Team, 2016)

Using LOADEST regression analysis with our relatively small dataset of TN values may introduce unnecessary error by basing flux calculations on weak relationships between N concentration and discharge. Because of this potential problem, we also estimated flux using average discharge and average N concentration. Only the calibration data (site 1 n=16, site 2 n=36) was used in this calculation so as not to use discharge data that lacked a corresponding concentration measurement and unintentionally skew the data.

RESULTS

In this study, we calculated total inputs as the sum of NANI and wildlife inputs in the form of wildebeest carcasses. As an area-weighted value, NANI in the Emarti basin was 2070.1 kg N km^{-2} yr^{-1} and was 1741.2 kg N km^{-2} yr^{-1} in the NMB basin (Table 1). The annual budget on a per area basis shows net imports of agricultural products as the dominant anthropogenic input, ranging from 47.3% to 52.8% (Table 2). Atmospheric deposition contributed the second most anthropogenic N (36.2% to 43.0%), followed by leguminous crop fixation at 8.8% to 10.1% of total inputs. Unlike other areas of the world, fertilizer application accounted for very little of the human inputs, contributing <1% of all human nitrogen inputs. Of the three inputs controlled by human and livestock population density (fixation, fertilizer, net imports), all were proportionately higher in the Emarti basin. In terms of wildlife inputs, nitrogen loading via wildebeest carcasses accounted for 0.1% of the inputs for NMB (Table 3). In looking at the potential variation in the NANI estimates, Emarti NANI ranges from 1000.2-2536.1 kg N km^{-2} yr^{-1} and NMB ranges from 812.8-2108.2 kg N km^{-2} yr^{-1} (Table 4)

We also calculated riverine N flux, and using flux and our input estimations, calculated a retention rate for each basin (Table 3; Figures 2-4). Flux was estimated three different ways: in LOADEST using two distinct methods as described previously, and as an average of simultaneous discharge and concentration samples. In the interest of simplicity, the two LOADEST models will hereafter be referred to as 'All Discharge' (estimate made using all discharge data) and 'Model Discharge' (estimate made using discharge data used to build the model), and the averaged data as 'Average'.

In LOADEST, Emarti flux was estimated at 15.0-18.7 Mg N yr^{-1}, and NMB flux was estimated at 56.7-81.4 Mg N yr^{-1}. Retention rate for the basins ranged from 99.3% to 99.7% of total inputs, with Emarti retaining slightly more annually. Using an average, Emarti flux was estimated at 490.9 Mg N yr^{-1} (retention rate of 90.3%), and NMB flux was estimated at 1690.0 Mg N yr^{-1} (retention rate of 85.1%).

DISCUSSION

Of the total NANI and wildlife inputs, flux accounts for 0.3-14.9%, meaning more than roughly 85-99% of the nitrogen is retained in the basin, either stored through biological uptake and sediment sorption or lost through denitrification as gaseous nitrogen (Figure 5) (Van Breemen et al., 2002). In comparison, the Lake Victoria Basin as a whole has a retention rate of 84%, while watersheds in the U.S. and Europe have a combined average of ~75% (Howarth et al., 1996; Zhou et al., 2014). Of the methods used to calculate flux, we believe that the average is the most accurate portrayal of the Mara Basin at this point in time, and that more concentration data will lead to a more reliable LOADEST model.

In our hypothesis we cited direct mobilization of terrestrial N stocks into the river as a major reason for wildlife presence decreasing N retention rates in the lower basin. Direct mobilization via litterfall in the Amazon has been found to significantly decrease retention rates, and hippos in the Mara are estimated to contribute roughly 18 Mg N yr^{-1} in the form of excreta (Howarth et al., 1996; Subalusky et al., 2015). Wildebeest contribute 13.2 Mg N yr^{-1} to NMB and the presence of wildlife reduced retention rate by 0.1-5.2% between the two basins. However, it's hard to say whether wildlife are the cause of this or if it's a combination of wildlife and other factors, which may include an increase in discharge or decreases in vegetative uptake, denitrification, or sediment sorption (Saunders and Kalff, 2001). In direct contrast with our hypothesis, wildlife also has the potential to increase N retention. As wildebeest and other ungulates migrate through the area, their grazing activities stimulate microbial activity and primary production in plants (Frank et al., 2000; McNaughton, 1976). The newly growing plant tissue is much higher in nutrient content than the recently grazed material, and both plant growth and microbial activity have the potential to increase N retention terrestrially. An increase in terrestrial retention would prevent some of the calculated inputs from ever reaching the hydrosphere.

In addition to natural influences on retention rate, human-contributed nitrogen in the Mara is different from many other places in the world. Fertilizer application in this region is incredibly low compared to more developed areas with easy access to fertilizers (Potter et al., 2010). Because of this, much of the nitrogen is absorbed by the plants and is not as susceptible to loss by leaching (Munoz et al., 2003). Additionally, many of the inputs to croplands in the basin are organic (i.e., manure, sewage, etc.), which can be stored in the soil for a number of years. In the term 'net imports of food and feed', production is a summarizing indicator of the intensity of agriculture, and consumption is related to human and livestock nutrition, namely the production of excreta (Billen et al., 2010). The Mara is considered a heterogenic basin, meaning consumption exceeds production and the net imports term is indicative of human and livestock wastes, and these wastes account for ~50% of the total inputs to both basins. Rural East Africa generally lacks infrastructure such as wastewater treatment and sewage systems, which are inherently leaky and sometimes connected directly to river systems (Kuroda et al., 2012). Because of this, waste in this region is most often incorporated into the nearby soil and only comes into contact with the river and groundwater over long periods of time (Maeda et al., 2003). Furthermore, the waste that quickly reaches the hydrosphere contains nitrogen in highly labile forms that are quickly absorbed by organisms, effectively removing this nitrogen from the flux measured downstream. Because of these anthropogenic and natural effects on retention rate, we can assume that the non-exported NANI and wildlife inputs are either stored in the soil or denitrified. However, there are no data on soil storage and denitrification rates in the basin, making it difficult to substantiate these assumptions.

Due to a great amount of variability in discharge and concentration measurements, there is a large amount of uncertainty in our estimation of flux. For example, the annual flux calculated in LOADEST for NMB varies between 33.7 and 131.2 Mg N yr^{-1} (95% confidence interval) depending on the method used. Our estimates for inputs are also prone to uncertainty due to a high degree of variability in references, resolution of spatial data, and the year in which the data was taken. Much of our data for agricultural and livestock production rates, atmospheric deposition, and human consumption came from studies conducted outside the Mara River Basin. Monitoring data on atmospheric deposition was taken as an average of other stations in similar ecosystems in Africa, but these may not reflect Mara deposition rates. Additionally, our spatial data for the basin varied widely in its resolution (0.083^{o} to 0.5^{o}) and did not conform precisely to the watershed boundaries, requiring us to make estimates that may not accurately reflect nitrogen loading in the basin. However, our estimate is likely in the ballpark because both basins are near the 95% confidence interval for worldwide trends (Figure 5).

This study is the first to examine nitrogen dynamics in the Mara River Basin, though others have looked at larger basins in East Africa, including Lake Victoria. Anthropogenic inputs to the basin were by far the

most important in determining total nitrogen load, accounting for >99% of the total inputs. Of the NANI components, net import of food and feed made up about half of the inputs to both basins. Because of the minimal use of fertilizer in the basin and the region in general, there could be a general degradation of soil nutrients that would severely limit current and future agricultural activities crucial to the people and the economy in the area. In addition, as infrastructure such as wastewater treatment and fertilizers become more accessible, careful management will be necessary to prevent increasing direct inputs to the river as has been seen in many other areas of the world. Though we have successfully estimated a nitrogen budget for the Mara, this estimate is not without uncertainty. To improve upon this, we suggest that 1) spatially uniform data be compiled on agricultural activities (crops and livestock) and 2) monitoring surveys of deposition rates in the basin be conducted. In addition to increasing our knowledge base in local human inputs, further investigation of wildlife's role in nitrogen cycling in East Africa is necessary to fully understand natural contributors to N cycling. Not only would investigations such as these help our understanding of the combined effects of humans and wildlife on ecosystem level nitrogen cycling in the Mara, but it could be extended to research in other areas in Africa and the world.

ACKNOWLEDGEMENTS

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APPENDIX

TABLE 1. Area-weighted NANI components for the Emarti and NMB basins.

Budgetary Item	Emarti (kg N/km^2/yr)	NMB (kg N/km^2/yr)
Atmospheric Deposition	750.00	750.00
Crop Fixation	208.27	153.44
Fertilizer Application	19.83	13.69
Net Imports	1090.98	823.37
Human Consumption (+)	*376.63*	*237.52*
Livestock Consumption (+)	*1245.85*	*972.74*
Livestock Production (-)	*374.90*	*276.67*
Crop Production (-)	*156.60*	*110.22*
NANI	**2069.08**	**1740.50**

TABLE 2. NANI inputs as a percent of the total inputs to each basin.

Budgetary Item	Emarti (% of Total)	NMB (% of Total)
Atmospheric Deposition	36.25	43.09
Crop Fixation	10.07	8.82
Fertilizer Application	0.96	0.79
Net Imports	52.73	47.31

TABLE 3. Total NANI and wildlife inputs (Mg N yr^{-1}) for the Mara River Basin using two different methods of estimating daily flux.

	All Discharge		Model Discharge	
Budgetary Item	**Emarti (Mg N/yr)**	**NMB (Mg N/yr)**	**Emarti (Mg N/yr)**	**NMB (Mg N/yr)**
Atmospheric Deposition	1840.1	4873.1	1840.1	4873.1
Crop Fixation	511.0	997.0	511.0	997.0
Fertilizer Application	48.7	88.9	48.7	88.9
Net Imports	2676.7	5349.8	2676.7	5349.8
Human Consumption (+)	*924.1*	*1543.3*	*924.1*	*1543.3*
Livestock Consumption (+)	*3056.7*	*6320.3*	*3056.7*	*6320.3*
Livestock Production (-)	*919.8*	*1797.7*	*919.8*	*1797.7*
Crop Production (-)	*384.2*	*716.2*	*384.2*	*716.2*
Wildebeest Carcass	NA	13.2	NA	13.2
INPUTS (Mg N/yr)	5076.5	11308.9	5076.5	11308.9
FLUX (Mg N/yr)	18.7	56.7	15.0	81.4
Retention Rate (%)	99.6	99.5	99.7	99.3

	Average	
Budgetary Item	**Emarti (Mg N/yr)**	**NMB (Mg N/yr)**
Atmospheric Deposition	1840.1	4873.1
Crop Fixation	511.0	997.0
Fertilizer Application	48.7	88.9
Net Imports	2679.3	5354.4
Human Consumption (+)	*924.1*	*1543.3*
Livestock Consumption (+)	*3056.7*	*6320.3*
Livestock Production (-)	*917.3*	*1793.1*
Crop Production (-)	*384.2*	*716.2*
Wildebeest Carcass		13.2
INPUTS (Mg N/yr)	5079.0	11326.6
FLUX (Mg N/yr)	490.9	1690.0
Retention Rate (%)	90.3	85.1

TABLE 4. Low and High estimates of NANI for both basins (kg N km^{-2} yr^{-1}).

	HIGH	
Budgetary Item	**Emarti (kg N/km2/yr)**	**NMB (kg N/km2/yr)**
Atmospheric Deposition	880.00	880.00
Crop Fixation	366.50	270.02
Fertilizer Application	19.83	13.69
Net Imports	1269.77	944.44
Human Consumption (+)	*376.63*	*237.52*
Livestock Consumption (+)	*1245.85*	*972.74*
Livestock Production (-)	*203.42*	*160.95*
Crop Production (-)	*149.30*	*104.87*
NANI	**2536.09**	**2108.15**

	LOW	
Budgetary Item	**Emarti (kg N/km2/yr)**	**NMB (kg N/km2/yr)**
Atmospheric Deposition	150.00	150.00
Crop Fixation	12.08	8.90
Fertilizer Application	19.83	13.69
Net Imports	818.29	640.20
Human Consumption (+)	*346.28*	*218.38*
Livestock Consumption (+)	*1245.85*	*972.74*
Livestock Production (-)	*608.64*	*434.41*
Crop Production (-)	*165.20*	*116.51*
NANI	**1000.19**	**812.78**

Erik Arndt (2015)

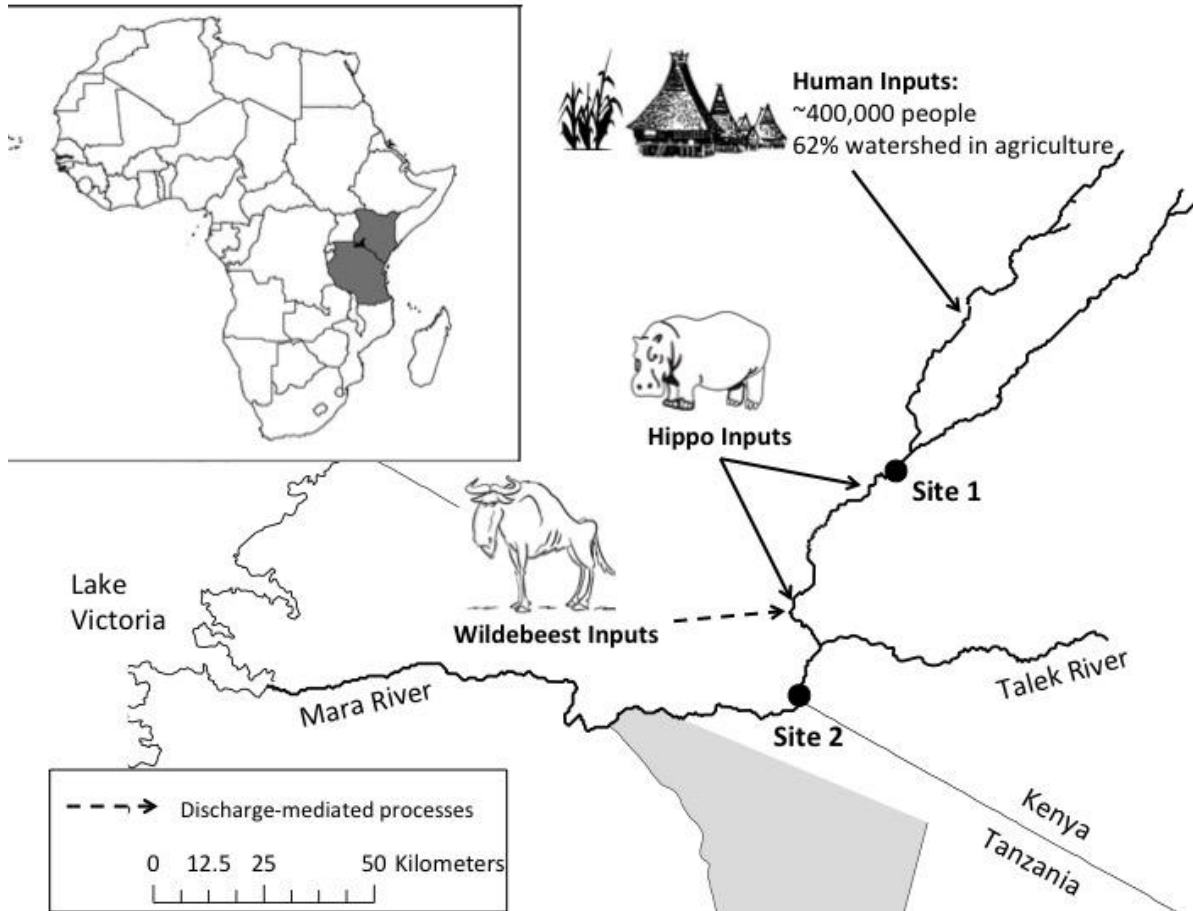**FIGURE 1.** Map of the Mara River Basin and testing sites for the study. Site 1 refers to Emarti and Site 2 refers to New Mara Bridge (NMB).
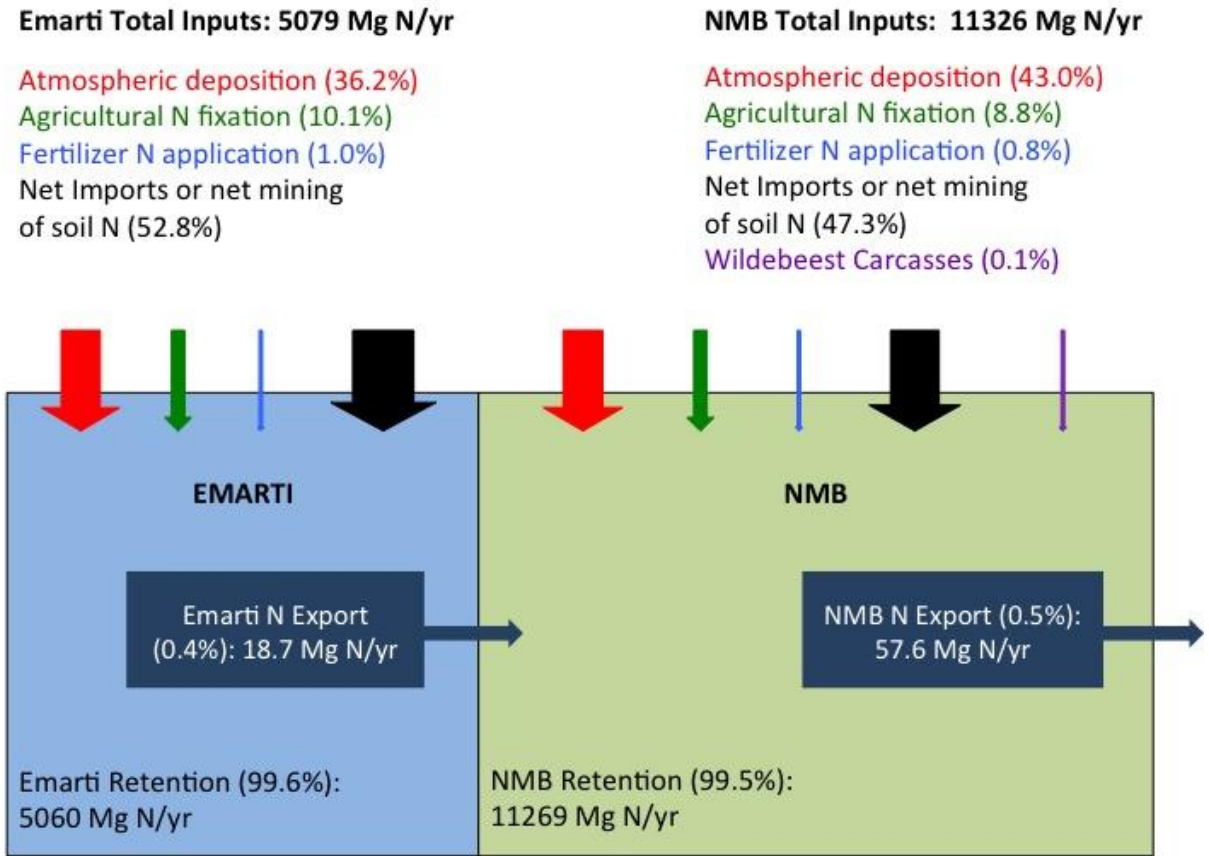
Emarti Total Inputs: 5079 Mg N/yr

Atmospheric deposition (36.2%)
Agricultural N fixation (10.1%)
Fertilizer N application (1.0%)
Net Imports or net mining
of soil N (52.8%)

NMB Total Inputs: 11326 Mg N/yr

Atmospheric deposition (43.0%)
Agricultural N fixation (8.8%)
Fertilizer N application (0.8%)
Net Imports or net mining
of soil N (47.3%)
Wildebeest Carcasses (0.1%)

EMARTI

NMB

Emarti N Export
(0.4%): 18.7 Mg N/yr

NMB N Export (0.5%):
57.6 Mg N/yr

Emarti Retention (99.6%):
5060 Mg N/yr

NMB Retention (99.5%):
11269 Mg N/yr

FIGURE 2. Flow diagram describing inputs, flux, and retention rate for both basins (Mg N yr^{-1}). Flux estimated using the All Discharge method.

Emarti Total Inputs: 5079 Mg N/yr

Atmospheric deposition (36.2%)
Agricultural N fixation (10.1%)
Fertilizer N application (1.0%)
Net Imports or net mining
of soil N (52.8%)

NMB Total Inputs: 11326 Mg N/yr

Atmospheric deposition (43.0%)
Agricultural N fixation (8.8%)
Fertilizer N application (0.8%)
Net Imports or net mining
of soil N (47.3%)
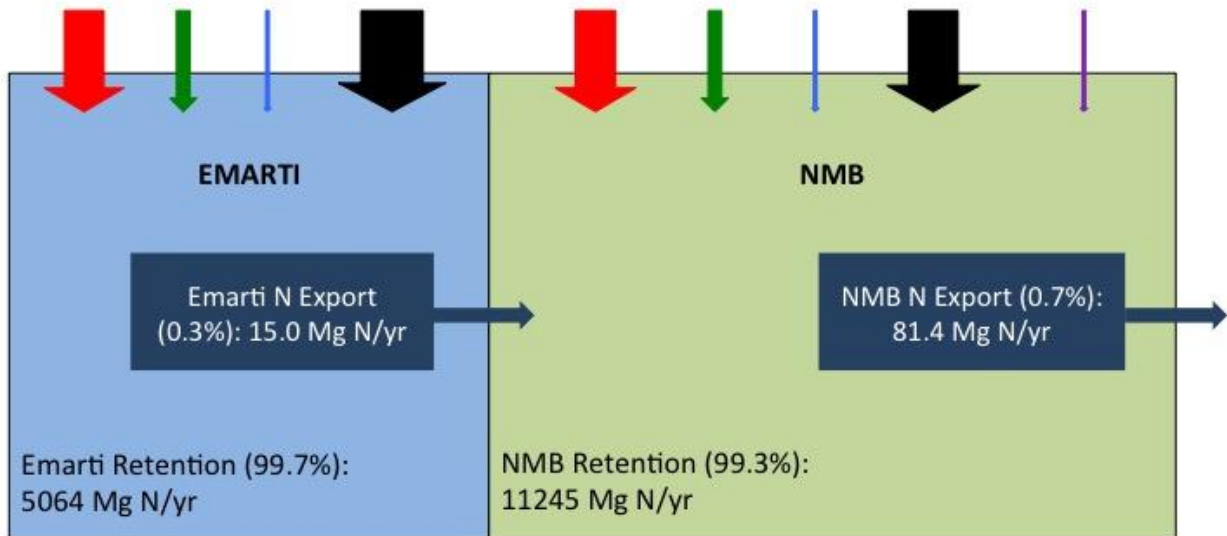Wildebeest Carcasses (0.1%)

EMARTI

NMB

Emarti N Export
(0.3%): 15.0 Mg N/yr

NMB N Export (0.7%):
81.4 Mg N/yr

Emarti Retention (99.7%):
5064 Mg N/yr

NMB Retention (99.3%):
11245 Mg N/yr

FIGURE 3. Flow diagram describing inputs, flux, and retention rate for both basins (Mg N yr^{-1}). Flux estimated using the Model Discharge method.

Emarti Total Inputs: 5079 Mg N/yr

Atmospheric deposition (36.2%)
Agricultural N fixation (10.1%)
Fertilizer N application (1.0%)
Net Imports or net mining
of soil N (52.8%)

NMB Total Inputs: 11326 Mg N/yr

Atmospheric deposition (43.0%)
Agricultural N fixation (8.8%)
Fertilizer N application (0.8%)
Net Imports or net mining
of soil N (47.3%)
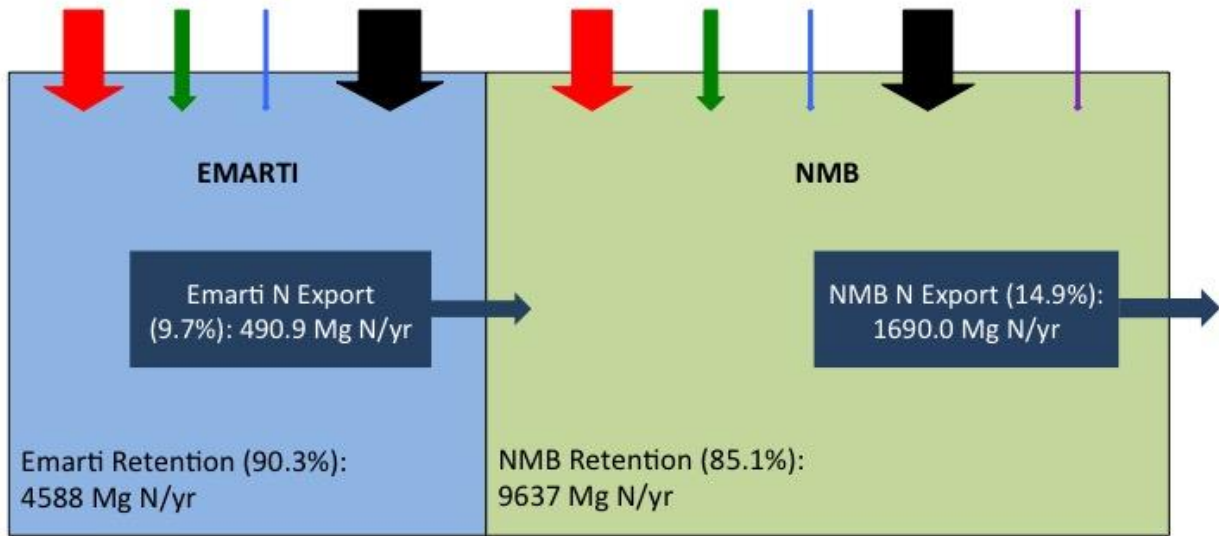Wildebeest Carcasses (0.1%)

EMARTI

Emarti N Export
(9.7%): 490.9 Mg N/yr

Emarti Retention (90.3%):
4588 Mg N/yr

NMB

NMB N Export (14.9%):
1690.0 Mg N/yr

NMB Retention (85.1%):
9637 Mg N/yr

FIGURE 4. Flow diagram describing inputs, flux, and retention rate for both basins (Mg N yr^{-1}). Flux estimated as an average of discharge and concentration.
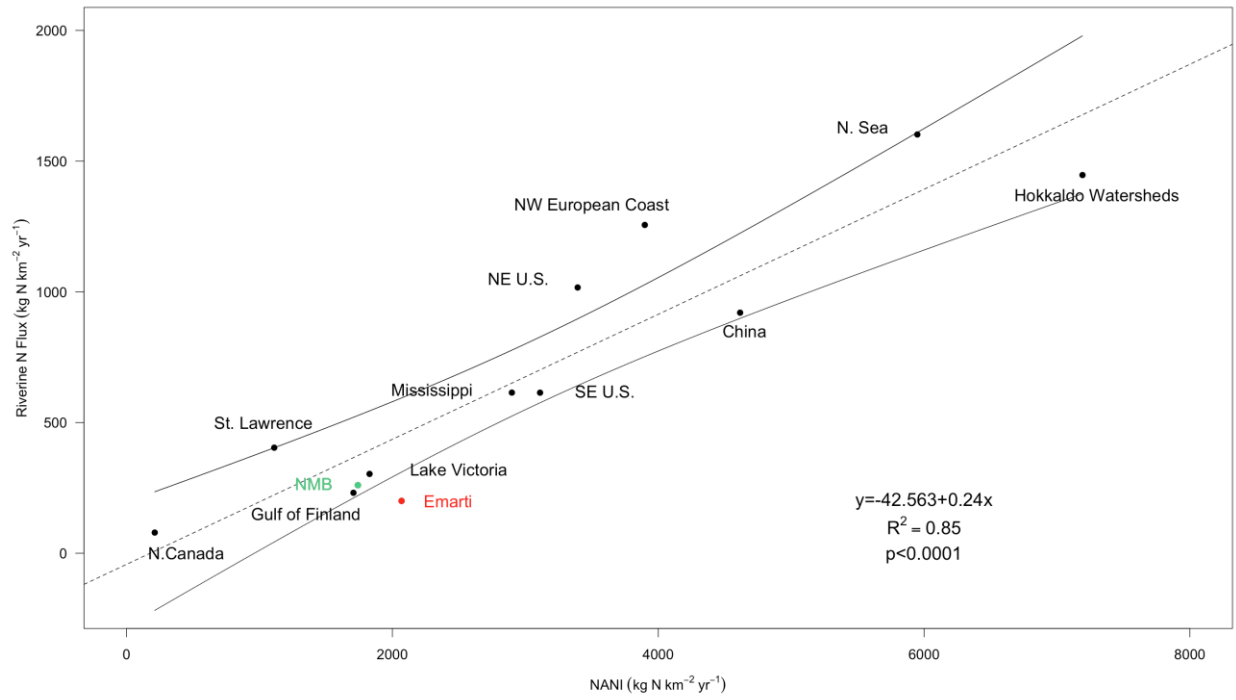
FIGURE 5. Plot comparing NANI and riverine N flux in watersheds around the world. Data sources: China from Ti et al. (2012), Hokkaido Watersheds from Hayakawa et al. (2009), Gulf of Finland from Hong et al. (2011), Lake Victoria from Zhou et al. (2014), all others from Howarth et al. (1996).