RESEARCH ARTICLE

Limited animal-facilitated nutrient transfer across an aquatic–terrestrial interface in a southern African savanna

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Animal-mediated nutrient transfer facilitates nutrient cycling in marine, freshwater and terrestrial ecosystems *via* the deposition of aquatically-derived nutrients such as nitrogen (N) and carbon (C). This mode of nutrient transfer has not been studied in southern African river systems. We investigated nutrient deposition associated with defecation, urination, and scent-marking at latrine sites of a semi-aquatic predator species (African clawless otters *Aonyx capensis*) in a riparian zone of a Bankenveld savanna ecosystem. We provide a comparison of stable nitrogen isotopes (δ^{15}N) measured in soil, vegetation and faecal material, between latrine and paired control sites. Latrine sites displayed higher δ^{15}N values than the paired non-latrine sites, but only at the area of direct deposition (soil surfaces). This effect dissipated as the distance from direct contact increased, while no significant difference in δ15N values was detected for sub-surface soil samples. Plants displayed varying trends of enriched δ15N values between the latrine and paired control sites. These results suggest that several factors and processes such as leaching, mineralization, ammonia volatilization, and nitrogen acquisition influence the nutrient availability within latrine-soil-vegetation systems in riparian zones of African savanna ecosystems.

Keywords: aquatic–terrestrial linkages, stable isotopes, riparian, African clawless otter, *Aonyx capensis*.

INTRODUCTION

Nutrients, detritus and prey move across ecosystem boundaries altering biogeochemical processes as well as community compositions, which influence population dynamics (Polis & Hurd, 1996). Theoretical models predict that nutrients move from areas of high productivity to areas of lower productivity, altering consumer–resource interactions within the receiving system (Polis & Hurd, 1996; Polis *et al*., 1997; Ellis *et al*., 2006). Nutrients transported along this productivity gradient are facilitated by physical or biotic vectors (Polis *et al*., 1997; Ellis *et al*., 2006). For example, animal-mediated nutrient transfer facilitates nutrient cycling in marine, freshwater and terrestrial ecosystems (Meyer & Schultz, 1985; Hjerne & Hansson, 2002; Vanni, 2002; Knight *et al*., 2005).

Animal-mediated nutrient cycling links aquatic and terrestrial systems *via* the deposition of aquatically-derived organic and inorganic nutrients such as nitrogen (N), carbon (C) and phosphorous (P) (Polis & Hurd, 1996; Polis *et al*., 1997; Crait & Ben-David, 2007). This occurs predominantly *via* animals feeding in marine or freshwater systems, followed by faecal deposition of inorganic nutrients (hereafter referred to as nutrients) in terrestrial systems (Polis *et al*., 1997). For example, seabirds (Ellis *et al*., 2006), sea turtles (Hannan *et al*., 2007; Vander Zanden *et al*., 2012) and brown bears (*Ursus arctos*) (Hilderbrand *et al*., 1999) fertilize terrestrial systems *via* the introduction of aquatic-derived nutrients to terrestrial systems. This nutrient deposition often leads to localized increases in primary production (Roe

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et al., 2010), as well as ecosystem modifications such as localized increases in biodiversity, changes in landscape heterogeneity, trophic level alterations and nutrient cycling (Ben-David *et al.*, 1998a,b; Helfield & Naiman, 2001; Reimchen *et al.*, 2003). Several studies have examined the role of animal-mediated nutrient transfer within ecosystems, but the majority focus on northern hemisphere systems (Ben-David *et al.*, 1998a; Hilderbrand *et al.*, 1999; Helfield & Naiman 2001; Atkinson *et al.*, 2017), with limited understanding of linkages between aquatic and terrestrial systems in the southern hemisphere (Atkinson *et al.*, 2014).

Semi-aquatic predators forage in aquatic systems, but spend most their time on land where they urinate, defecate and occasionally scent-mark at communal latrine sites (Rowe Rowe, 1992; Ben-David *et al.*, 1998a; Crait & Ben-David 2007; Roe *et al.*, 2010). Scent-marking is seemingly achieved through secreting an anal jelly-like substance that is often coupled with urination and defecation (Jordaan *et al.*, 2017), thereby depositing nitrogen derived from their aquatic prey. Ben-David *et al.* (1998a) investigated the impact of river otter (*Lutra canadensis*) latrine sites on terrestrial vegetation at aquatic–terrestrial borders. These authors found that river otters transfer aquatically-derived nitrogen to the terrestrial system, fertilizing the terrestrial vegetation. Another study investigated plant growth and nitrogen content at river otter latrine *versus* non-latrine sites using stable nitrogen isotopes (δ^{15}N) (Roe *et al.*, 2010). These authors found that the dominant tree and shrub species assimilated aquatically-derived nutrients deposited at latrine sites (Roe *et al.*, 2010). Additionally, the plant responses to river otter activity (increased leaf-tissue concentrations of nitrogen, increased plant production, and/or disturbance susceptibility) depended on the plant species as well as the biological level of organization (*i.e.* leaf, plant or ecosystem) (Roe *et al.*, 2010). These North American studies (Ben-David *et al.*, 1998a; Roe *et al.*, 2010) did not include seasonality. Significantly, prey availability and diet of semi-aquatic predators in southern African systems vary seasonally (Somers, 2000; Somers & Nel, 2003; Jordaan *et al.*, 2015), and will likely influence nutrient content and amount available for animal-facilitated nutrient transfer.

Animal-facilitated inorganic nutrient transfer across the aquatic–terrestrial interface of freshwater systems has not, to the best of our knowledge, been studied extensively in southern African savanna systems. Semi-arid, bushveld savanna systems such as the Bankenveld have characteristically shallow, nutrient-poor and leached soils (Acocks, 1953; Bredenkamp & Brown, 2003). Ben-David *et al.* (2005) proposed that communal latrines could become potential inorganic nutrient sinks receiving substantial quantities of limiting nutrients (*e.g.* nitrogen). Given our limited understanding of nutrient transfer in communal latrine systems in southern African savannas, we aimed to determine if similar nutrient addition occurs in these nutrient-limited systems.

Nitrogen and carbon isotopes occur naturally in the environment, and the stable ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ have become widespread elements used as tracers in ecological research (Ben-David *et al.*, 1988a; Roe *et al.*, 2010). These isotope ratios are particularly useful to animal ecologists because differences in these ratios are indicators of nutritional origin within food webs, as well as the spatial origin between isotopically distinct food webs (*e.g.* freshwater *vs* terrestrial) (Hobson, 1999).

We investigated the role of semi-aquatic predators in transferring aquatically-derived nutrients to a terrestrial system through defecation, urination, and scent-marking in a southern African bushveld environment. Stable nitrogen isotopes (δ^{15}N) and percentages were measured in soil, plant and faecal material during summer and winter to determine whether latrine sites utilized by African clawless otters (*Aonyx capensis*) (and occasionally water mongoose, *Atilax paludinosus*) have significantly different δ^{15}N and %N values compared with paired non-latrine sites. Soil samples at latrine sites were predicted to have enriched δ^{15}N values and higher %N values at areas of direct faecal deposition due to leaching of aquatically-derived N from faecal material. Vegetation samples within latrine site were also predicted to have δ^{15}N and %N values reflective of the aquatic N. The overall influence of aquatically-derived N was expected to decrease with increased distance from the latrine centres.

MATERIALS AND METHODS

Study area

Otter faecal samples, and soil and vegetation samples were collected along the Wilge River bank between Telperion Nature Reserve and Ezemvelo Nature Reserve (25.7084°S, 28.9297°E)

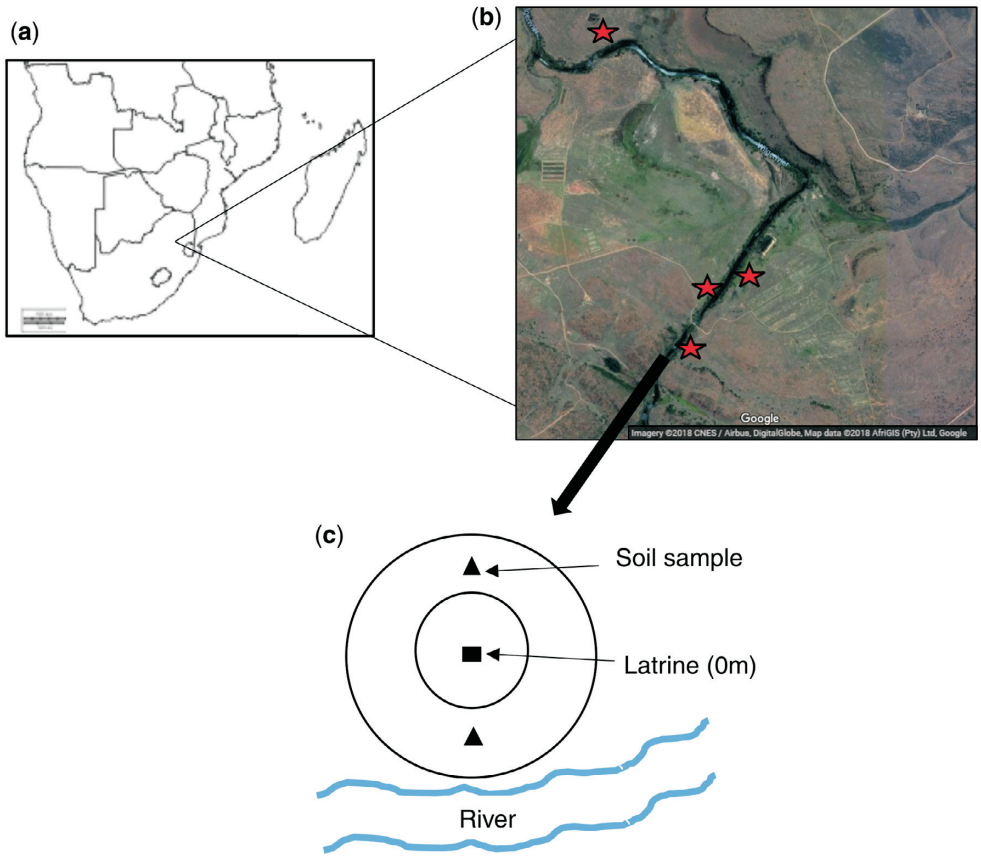

Fig. 1. The location of our study site within southern Africa (**a**). Sampling took place on the Telperion Nature Reserve side of the Wilge River, which runs between the Ezemvelo Nature Reserve and Telperion Nature Reserve (**b**). The latrine (red stars) and paired control sites were sampled according to the idealized scheme (**c**).

in South Africa (Fig. 1). The two conservation areas combined are 11 000 ha in size. We chose these conservation areas as they are relatively undisturbed and representative of the natural system. Additionally, the presence of semi-aquatic predators along the Wilge River, as well as the use of latrine sites along the river is well established (Scott, 2014). This area receives most rain during the summer months, peaking between October to March, with the driest months falling during winter, between June and August (Bredenkamp & Brown, 2003). This area is classified as Bankenveld (Acocks, 1953) based on the occurrence of characteristically nutrient-poor and sandy soils. Vegetation types are characteristic of open savannas, where much of the bushveld vegetation has changed to grassveld vegetation maintained by regular intervals of fires (Acocks, 1953; Bredenkamp & Brown, 2003).

Otter latrines and faecal material were identified based on the shape and size of faecal material, and the characteristic smell thereof (Stuart & Stuart, 2000). Otter latrine sites in our study area are known to be used on occasion by water mongoose (Somers pers. obs.). Therefore, while we are confident that African clawless otters were responsible for creating the sampled latrines, we could not exclude the possible influences of water mongoose. For the purposes of our study, four latrine sites were paired with four control sites. The control sites were selected on the basis of no direct observations of otter presence, as well as the lack of tracks or residual faecal material. These sites all approximated the latrine sites in physical, vegetative and topographic characteristics, including distance from the river, slope angle and vegetation cover.

Sampling protocol

Samples of vegetation, soil and otter faecal

material (at latrine sites) were collected at four latrine and four control sites during summer and winter. Materials were collected from the centre of each site (0 m), as well as a distance of 2 m away from the centre in both up- and downslope directions from the adjacent river. Vegetation samples were collected from the most common species (*Celtis africana*, *Dicliptera extenta*, *Diospyros lycioides*, *Gymnosporia buxifolia*, *Achyranthes aspera* and *Poaceae* sp.) occurring at each site. Approximately 25 g of young leaves and/or new growth from at least one grass, shrub and tree species common to all sites was collected at each of the sites. Where possible, vegetation samples were collected from five individual plants per species at each location. The stems were separated from the leaves, after which the leaf material was dried overnight at 70°C. The dried plant material was homogenized using a micro-tube homogenizer (Beadbug, supplied by Lasec, SA).

Approximately 50 g of soil was collected at varying depths (surface, 5 cm, 10 cm and 15 cm) from each sampling point at each site ($n \approx 12$ per site). Soil samples were sieved and homogenized using a mortar and pestle and subdivided into two equal batches. To avoid interference with the measurements of organic ^{13}C in the soils, the first batch was treated with a 1% HCl (0.5 molar) wash to remove any inorganic carbonates from the soil. The soil samples were repeatedly washed with 1% HCl to ensure the complete removal of any inorganic carbonates. After the acid treatment, soil samples were rinsed with distilled water five times, to ensure the removal of all excess acid. The second batch was left untreated. All soil samples were dried in an oven at 70°C, for 2–3 days, sieved again and homogenized using a mortar and pestle.

The faecal samples (total faeces $n = 9$) were dried at 70°C, for 2–3 days and subdivided. One sub-sample was left untreated and the other acid washed with 1% HCl (0.5 molar), then rinsed repeatedly with distilled water and dried. Nitrogen isotope and percentage values were obtained from untreated soil and faecal samples.

Aliquots of plant (1.0 to 1.1 mg), soil (10 to 15 mg) and faecal (0.5 to 0.6 mg) samples were weighed into tin capsules, pre-cleaned with toluene. All isotopic analyses were carried out using a Flash Elemental Analyser (EA 1112 Series) coupled *via* a ConFlo IV system to a Delta V Plus stable light isotope ratio mass spectrometer (all equipment supplied by Thermo Fischer, Bremen, Germany). These are housed at the Stable Isotope Laboratory, Mammal Research Institute, University of Pretoria.

A blank sample and a calibrated standard (Merck Gel: $\delta^{15}N = 6.5‰$, N% = 14.64) were analysed after every 12 study specimens. The isotope values were normalized to this internal running standard (10 replicates per run). All results were referenced to air for nitrogen isotopic values. Results are expressed in delta notation using a per mill scale using the standard equation:

$$\delta X = \left[\frac{\text{Rsample}}{\text{Rstandard}} - 1 \right]$$

where X = ^{15}N and R represents $^{15}N/^{14}N$.

A sample with a negative δ-value represents less of the heavy isotope in the sample relative to the standard, whereas a positive δ-value represents more of the heavy isotope relative to the standard (Sulzman, 2007). The precision for $\delta^{15}N$ was <0.08‰.

Statistical analysis

All statistical tests were run in the R programming environment (R Core Team 2015) using the R Studio (version 3.2.3) interface. A Wilcoxon sign test was used to test for significant differences between the latrine sites and paired control sites. This was done for $\delta^{15}N$, and %N values from both soil and vegetation samples between sites, at depths and at their relative positions (centre, upslope and downslope). An α level of 0.05 was used to indicate statistical significance on all tests.

The processes taking place within the site, within the soil column, between different plant species, as well as the soil–vegetation interactions as a result of the faecal material deposited at the latrine sites were visualized through the construction of plots using the R Studio interface of R.

RESULTS

Soil samples

Isotopic data from the individual latrine and control sites were pooled at each location (*i.e.* centre, upslope and downslope) into combined latrine or control sites, respectively, to account for any differences caused by physical, vegetative and topographic characteristics of individual sites. The variation in isotopic data between individual latrine sites were not significant for soil and vegetation samples collected at varying depths (Table S1) and distance (Table S2) from the latrine

centre ($p > 0.05$). Soil surface δ^{15}N values (0 cm) were significantly higher at latrine sites ($P < 0.05$, Fig. 2), but δ^{15}N values at depths below the surface (5 cm, 10 cm, 15 cm) were not significantly different between latrine and paired control sites during summer (Fig. 2). The surface soil δ^{15}N measured at the centre of latrine sites during summer were significantly higher than the upslope- and downslope sampling locations that were not characterized by any increase in δ^{15}N (Fig. 3). The soil samples collected in winter did not differ significantly between latrine and control sites at the surface (*P*-value = 0.95, W = 140), at depths (5 cm: *P*-value = 0.74, W = 134; 10 cm: *P*-value = 0.79, W = 143; 15 cm: *P*-value = 0.81, W = 36), between the centre of the latrine sites and the upslope- or downslope sampling locations, or overall (*P*-value = 0.95, W = 1704) (Fig. 2). Nitrogen concentration (%N) also did not differ significantly ($P > 0.05$) with depth or distance from the centre of the latrine compared to control sites during summer and winter.

Vegetation samples

The δ^{15}N values of *Dicliptera extenta* and *Celtis africana* foliage were significantly higher on control sites compared to latrine sites during summer. Foliage samples collected during summer for *Diospyros lycioides*, *Gymnosporia buxifolia* and *Achyranthes aspera* did not differ significantly in δ^{15}N values between latrine sites and paired control sites (Table 1). Measurements of δ^{15}N during winter were not significantly different between latrine sites and control sites for any of the species sampled (Table 1). Nitrogen concentrations (%N) were significantly elevated in *D. lycioides* at latrines compared to control sites during summer (Table 1). However, nitrogen concentrations (%N) did not differ significantly for any of the other plant species sampled between latrine and control sites during summer or winter (Table 1).

DISCUSSION

Our results confirmed some facilitation of aquatically-derived inorganic nutrient transfer by semi-aquatic predators to terrestrial, riverine systems *via* their latrine sites. We found a gradient of fertilization, with highly localized nitrogen inputs in areas of direct faecal deposition, dissipating rapidly as the distance from the area of direct contact increased during summer. A pattern of decline with distance would be expected, and is consistent with the literature on aquatic–terrestrial nutrient transfer (Ben-David *et al*., 1998a,b; Hilderbrand *et al*., 1999; Roe *et al*., 2010). The δ^{15}N values were highest in the surface soil layers of latrine sites, with no significant differences observed between sub-surface soil samples from the latrine and control sites. Such increased values of δ^{15}N were further restricted to the centre parts of latrines, with no increases detected at distances of 2 m up- or downslope of the latrine centres during summer. The corresponding %N did not differ significantly at depths or distance from the centre of the latrine compared to control sites.

The lack of strong relationships between δ^{15}N values at latrine or control sites that we report contrast the findings for latrine sites of North

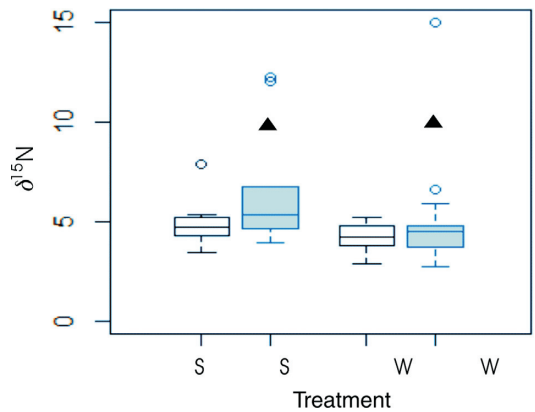

Fig. 2. Values of δ^{15}N for combined surface soil samples collected during summer (S) and winter (W) at latrine (light blue, *n* = 10 summer and 19 winter) and control (white, *n* = 10 summer and 16 winter) sites, respectively. Each box summarizes the interquartile range, the horizontal black line is the sample median, the whiskers represent 1.5 × interquartile range and the open circles are outliers. The average faecal δ^{15}N (black triangle) value for summer was 9.1‰ and for winter 9.4‰.

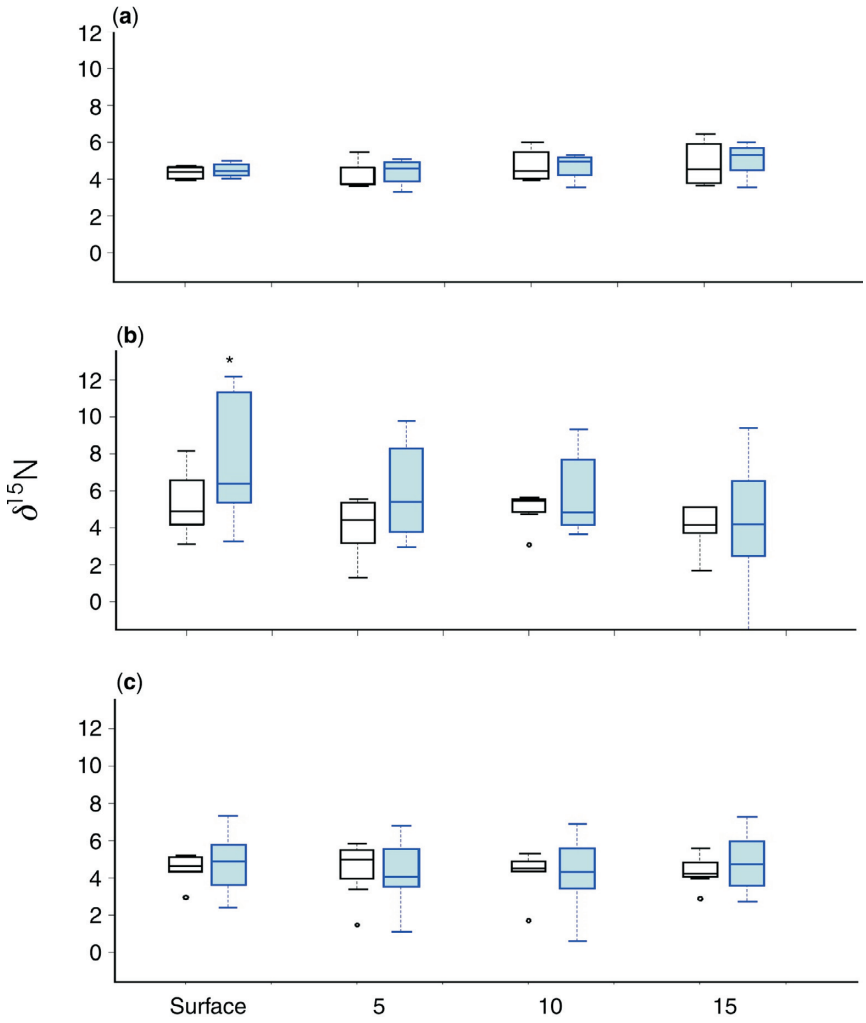

Fig. 3. Stable isotope values (δ^{15}N) at depths (0 cm, 5 cm 10 cm and 15 cm) for soil samples collected at the upslope (**a**, $n = 7$ paired), centre (**b**, $n = 16$ paired) and downslope (**c**, $n = 15$ paired) of both latrine (light blue) and control sites (white). The interquartile range is summarized by each box, the horizontal lines are sample medians, the whiskers represent 1.5 × interquartile range and the open circles are outliers.

American river otters (Ben-David *et al.*, 1998a, 2005; Crait & Ben-David, 2007; Roe *et al.*, 2010). In the case of the North American river otters, nutrient dynamics at latrine sites were altered by otter activity, with consistently elevated δ^{15}N values reported in plant species occurring in close proximity to otter latrines (*e.g. Picea sitchensis*, *Sambucus racemose* and *Pinus* sp.). Consistent with the North American studies (Ben-David *et al.*, 1998b; Hilderbrand *et al.*, 1999) were the dissipating effects of nutrient enrichment as distance from the area of direct deposition increases.

Both inconsistent and consistent trends between the North American studies and our study are likely due to several biological and biogeochemical processes. Firstly, deposition of N by otters may be displayed through indirect soil processes [*e.g.* microbial activity (Hilderbrand *et al.*, 1999)]; with direct fertilization by N playing a lesser role (Hilderbrand *et al.*, 1999; Crait & Ben-David, 2007). Secondly, sites receiving larger inputs of urinary or faecal deposition may not correlate with greater N availability to plants due to N cycling processes such as soil leaching, mineralization, volatilization and immobilization (Pastor *et al.*, 1997; Ben-David *et al.*, 2005; Crait & Ben-David, 2007).

Soil samples were collected from the Bankenveld habitat, a characteristically shallow, nutrient-

Table 1. Summary values (median) for δ^{15}N, and %N measured in soil depths and plant species from combined latrine and paired control sites during summer. Wilcoxon paired test results are also presented (statistically significant differences between latrines and control sites are highlighted in bold). *D. extenta* = *Dicliptera extenta*; *D. lycioides* = *Diospyros lycioides*; *C. africana* = *Celtis africana*; *G. buxifolia* = *Gymnosporia buxifolia*; *A. aspera* = *Achyranthes aspera*.

		Latrine			Control				
	Sample	*n*	δ^{15}N (‰)	%N	*n*	δ^{15}N (‰)	%N	*P*-value	*W*-value
	Faeces	9	6.5	1.40					
Soil (cm)	Surface	20	5.0	0.27	10	4.7	0.26	**0.04**	48
5		20	5.0	0.18	10	5.1	0.23	0.68	56
10		20	4.9	0.12	10	4.8	0.17	0.50	35
15		20	4.3	0.09	10	4.2	0.15	0.56	34
Vegetation	*D. extenta*	24	5.9	3.64	8	7.6	3.38	**<0.01**	19
	D. lycioides	31	1.1	2.92	23	0.8	2.27	0.14	444.5
	C. africana	5	2.6	2.96	8	4.1	2.74	**0.05**	6
	G. buxifolia	8	5.3	2.78	7	3.1	2.85	0.09	43
	A. aspera	13	3.6	3.16	6	8.8	2.87	0.15	22

poor soil group, with high levels of leaching (Bredenkamp & Van Rooyen, 1998; Bredenkamp & Brown, 2003). The high leaching properties likely result in N deposited *via* faecal material only being available for limited periods. A short period is created in which nutrient-poor soils can retain the aquatically-derived nutrients deposited at latrine sites. The period of availability is further shortened by ammonia volatilization, a process whereby organic forms of nitrogen (*e.g.* urea, the main source of N in faecal material) are subject to high levels of volatile losses (Ernst & Massey, 1960). Urea, or more specifically the NH_4 derived from urine, is readily converted and lost to the atmosphere *via* volatilization, resulting in lowered levels of N being retained within the soil (Ernst & Massey, 1960; Mizutani *et al.*, 1986; McFarlane *et al.*, 1995; Ben-David *et al.*, 1998a). Subsurface-soil and vegetation species therefore have limited time to acquire and utilize nitrogen from faecal deposits. Characterization of the soil chemistry was outside the scope of this initial study, but future studies incorporating this aspect during the sampling process will provide insight into how nutrients are incorporated and assimilated in the soil.

The data for our six plant species did not show similar patterns in δ^{15}N values at latrine or control sites. Two of the five tree/shrub species (*D. extenta* and *C. africana*) displayed significantly higher δ^{15}N values at the control sites compared to the latrine sites in summer. Only *D. lycioides* had significantly higher nitrogen concentrations at latrine sites during summer. In our system, we propose that the aquatically-derived nutrients in soils at latrine sites are not utilized by the vegetation. We further suggest that the inconsistent trends in δ^{15}N and %N levels in plants are not due to aquatically-derived nutrient transfer, but rather a combination of natural and physiological factors. Different plant species acquire and utilize nitrogen in different ways depending on differences between external and internal plant nitrogen concentration (Evans, 2001). The physiological mechanisms activated under different nitrogen concentrations include the pathways of nitrogen assimilation [*e.g.* fractionation with NH_4^+ and NO_3^- (Yoneyama *et al.*, 1991)] and/or the recycling of nitrogen (Evans, 2001). The physiological factors discussed provide a possible explanation for the inconsistent trends between plant δ^{15}N values at latrine and control sites. Other factors affecting δ^{15}N levels in plants include plant phenology, mycorrhizal effects, soil depth where individual plants can extract N, form of assimilated N and the tissue allocation of N (Hogberg, 1997; Evans, 2001; Crait & Ben-David, 2007). Additionally, there may be an associated disturbance effect by semi-aquatic predators utilizing these sites (Roe *et al.*, 2010).

Faecal material is not the only source of N deposited by semi-aquatic mammals. According to Hilderbrand *et al.* (1999), most nitrogen deposited by carnivores is likely to be in urine, which is rapidly converted to ammonium. Urinary deposition is not easily detected in the field, as otters and other semi-aquatic predators occasionally urinate

at sites without defecating (Reed-Smith *et al.*, 2014). It is possible that sites with fewer faecal samples actually receive larger N inputs from urinary deposits (Crait & Ben-David, 2007) and ammonium from urinary deposition may lead to enhanced N mineralization (Hobbs, 1996). The use of faecal material as an indication of nutrient deposition by otters may therefore be biased if the majority of N deposited is in the form of urea (Hilderbrand *et al.*, 1999). Future long-term research could barricade the control areas before and during the study to prevent otters from urinating in these areas. Latrine and non-latrine sites were in close proximity and selected to best approximate one another in terms of vegetation cover, slope angle, slope direction and topography. We therefore do not consider any of these variables as contributing factors to the differences in δ^{15}N values reported here for the soil samples. Due to no significant differences between the centre (area of direct deposition), upslope and the downslope positions, the possibility of a slope effect influencing the movement of nutrients is also not likely (Fig. 2 and Table S2).

The most likely factor influencing the contrasting results between summer and winter is the difference in availability of water (Ehleringer & Dawson, 1992). In savanna systems, the availability of water in the soil has been demonstrated to strongly influence the δ^{15}N values of soils and plants (Handley, Odee & Scrimgeour, 1994). Therefore during the dry winter seasons plant and soil δ^{15}N values may be substantially different to those recorded during the wet summer seasons. An additional factor possibly contributing to the contrasting results between summer and winter is the age and visitation rates of the latrines. No previous history of the use of the latrine sites were available for this study area, making it impossible to assess their age, although the presence of clearly older faecal material, as well as recent material, suggested that they were at least well established latrine sites. Older latrine sites may have more of an effect on the plant and soil δ^{15}N values due to relative faecal decomposition rates, N mineralization and mobilization as well as the usage histories of the latrine sites (Chang & Handley, 2000). Rate of deposition may also have an effect on plant and soil δ^{15}N values (Hogberg, 1997).

Moreover, faecal deposition is not uniformly distributed across latrine sites, while plant species may also have patch-like roots distributions. In combination, these two factors may account for greater variation between plant species within a latrine site than the control site (Ben-David *et al.*, 1998a). Larger latrines may possibly yield different results as more faecal material may be spread over a larger area, as well as potentially providing larger nutrient input to the terrestrial system.

CONCLUSION

Our results suggest that otters in African savanna systems, specifically the Bankenveld, contribute to processes of nutrient cycling *via* deposition of aquatically-derived nitrogen in soils but the uptake thereof is not realized in the vegetation. This forms part of a complex cycle made up of several processes such as leaching, volatilization and acquisition. The benefits derived from the aquatically-derived nutrients are highly localized with dissipating effects from the areas of direct deposition. We suggest that aquatically-derived nutrients made available in soils are not being utilized by the surrounding vegetation and are of no apparent benefit to plant growth or ecosystem productivity. The role that semi-aquatic predators play in facilitating nutrient transfer of limited nutrients in the Bankenveld was not substantial, and thus we suggest that aquatic nutrients are acquired in this terrestrial system through alternative pathways. This pattern of limited nutrient transfer may be specific to the Bankeveld, or sandy savanna systems studied here, and the role of aquatic predators in transferring nutrients in other systems (*e.g.* loamy or clay) across southern Africa may be more similar to those reported in the Northern American studies. Further analyses may indicate possible benefits that these semi-aquatic predators provide for plants at multiple levels (*e.g.* plant tissues, leaves, roots and stem), various growth forms (grasses, shrubs and trees) and for the soils. The potential disturbance to plant assemblages that may be associated with latrine usage should be incorporated into future studies aiming to quantifying the ecosystem productivity at latrine sites.

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Supplementary material to:

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Supplementary material

Table S1 Kruskal–Wallis test results for variation in soil and vegetation δ^{15}N values between latrine sites collected at various depths (0 cm, 5 cm, 10 cm and 15 cm) during summer. *D. extenta* = *Dicliptera extenta*; *D. lycioides* = *Diospyros lycioides*; *C. africana* = *Celtis africana*; *G. buxifolia* = *Gymnosporia buxifolia*; *A. aspera* = *Achyranthes aspera*.

	Sample	P-value	χ^2	df
Species	Soil	0.98	0.16	3
	D. extenta	0.74	0.60	2
	D. lycioides	0.02	7.61	2
	C. africana	0.50	1.4	2
	G. buxifolia	0.77	0.08	1
	A. aspera	0.33	2.23	2

Table S2 Kruskal–Wallis test results for variation in soil and vegetation δ^{15}N values between latrine sites collected at distances from the latrine centre (upslope, centre and downslope) during summer. *D. extenta* = *Dicliptera extenta*; *D. lycioides* = *Diospyros lycioides*; *C. africana* = *Celtis africana*; *G. buxifolia* = *Gymnosporia buxifolia*; *A. aspera* = *Achyranthes aspera*.

	Sample	P-value	χ^2	df
Species	Soil	0.50	1.37	2
	D. extenta	0.74	0.60	2
	D. lycioides	0.02	7.61	2
	C. africana	0.50	1.4	2
	G. buxifolia	0.77	0.08	1
	A. aspera	0.33	2.23	2