

THE RELATIONSHIP BETWEEN BIODIVERSITY AND NUTRIENT RECYCLING
BY SUBTERRANEAN INVERTEBRATES IN THE EDWARDS AQUIFER

by

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A thesis submitted to the Graduate Council of
Texas State University in partial fulfillment
of the requirements for the degree of
Master of Science
with a Major in Aquatic Resources
August 2017

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ACKNOWLEDGEMENTS

I would like to thank Dr. Weston Nowlin for all of his time and help assisting me with the research and writing of this thesis. I would also like to thank all of my committee members, Dr. Benjamin Schwartz, Dr. Todd Swannack, and Dr. Thom Hardy for aiding me throughout this process.

This research required a considerable amount of historical data and collection and I would like to thank Dr. Benjamin Schwartz, Pete Diaz, and Randy Gibson, for their help with field sampling and identification of organisms. I would also like to thank Gaby Timmins and Aaron Swink for training me in the many lab techniques and procedures required for this research.

Lastly, I would like to thank my friends and family, especially my mother and father, for supporting me in my education every step of the way.

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I. INTRODUCTION

For the past 25+ years, ecologists have examined and debated the relationship between ecosystem functioning and biodiversity (Tilman 1999; Loreau et al. 2002; Naeem et al. 2002; Balvanera et al. 2006; Loreau 2009; Vaughn 2010; Hooper et al. 2005). The biodiversity – ecosystem functioning hypothesis posits that if there is a reduction in local diversity (i.e., loss of species, genotypes, etc.), then ecosystem functioning will diminish as well (Srivastava and Vellend 2005). In particular, studies have found that the functional roles of species within a community strongly affect ecosystem processes and that maintenance of biodiversity within communities can affect ecosystem functioning in that it provides functional diversity related to the ecosystem processes of interest (Loreau 2009; Hooper et al. 2005). Thus, ecologists have argued that in light of current global biodiversity losses, there may also be a concurrent large-scale loss of ecosystem function and ecosystem services (Vaughn 2010). Despite the efforts by ecologists to understand the relationship between biodiversity and ecosystem functioning, there are still substantial gaps in our knowledge of how ecosystem functioning varies with changing biodiversity in ecosystems (Vaughn 2010; Loreau 2009).

Over 7,000 species of subterranean aquatic invertebrates have been described globally, with at least 700 of these species found across almost 400 counties in the United States alone (Culver et al. 2000; Botosaneanu 1987). Stygobionts (obligate subterranean aquatic organisms) represent diverse assemblages with an endemism rate (based on species that occur in only one county) of nearly 45% in the United States (Culver et al. 2000). Despite the widespread distribution and apparent unique evolutionary lineages of

these stygobionts, their role in ecosystem services and function is comparatively understudied. On a global scale, subterranean invertebrate communities in the Edwards Aquifer, a large karst system in central Texas, are among the most diverse in the world (Longley 1981). As such, the impact of these invertebrate assemblages on the biogeochemical processes of the Edwards Aquifer is investigated here to increase our understand of their effects on water quality and nutrient dynamics in order to inform management decisions aimed at water quality protection.

Metazoan organisms influence the overall chemical composition and distribution of biomass within an ecosystem through participating in the flux, storage, and turnover of chemical elements (Allen and Gillooly 2009). Recycling of inorganic nutrients by animals via excretion is an important process in ecosystems, affecting the metabolism of primary producers within the system and the species composition, by supplying limiting nutrients (Allen and Gillooly 2009). Aquatic invertebrate assemblages influence their ecosystem directly and indirectly via primary productivity, nutrient cycles, decomposition rate, and translocation of these materials within an ecosystem (Wallace and Webster 1996). Nitrogen (N) and phosphorous (P) excretion by invertebrates, as well as their aggregate ratio of excreted nutrients (i.e., N:P), is the most appropriate way to evaluate their effect on biogeochemical processes. Recycling of inorganic dissolved nutrient forms by aquatic metazoan excretion can supply resources for bacteria and primary producers, potentially influencing the degree of nutrient limitation and which particular element most limits primary and microbial production.

In subterranean and groundwater systems, there are no in situ photoautotrophic primary producers. Thus, these environments are thought of as being resource and energy

limited with a reliance upon allochthonous surface organic matter (OM) inputs of plant detritus, surface animal carrion, and bat guano (Simon and Buikema 1997; Graening and Brown 2003; Schneider et al. 2011) or on in situ chemosynthetic bacterial production (Sarbu et al. 1996; Roach et al. 2011; Hutchins et al. 2016). In addition, nutrients such as N and P can enter subterranean systems as dissolved inorganic forms (e.g., PO_4^{3-} , NO_3^-) via hydrological inputs from the surface or through weathering of geological substrates. The degree to which subterranean ecosystems are “open” to inputs of allochthonous OM and nutrients is in fact a continuum, with some systems being more open to inputs and some systems being relatively “closed” to direct inputs of OM and nutrients. In subterranean aquatic ecosystems with little or no direct and immediate connection to the surface, the relative importance of in situ recycling of OM and nutrients is likely to be much greater for their biotic communities (Simon and Benfield 2002; Engel et al. 2010).

Through the examination of how nutrient recycling (via excretion) by subterranean invertebrate communities change with differing levels of diversity I was able to directly link an ecosystem function (nutrient recycling rates and ratios) to biodiversity (*sensu* McIntyre et al. 2008). Indeed, several studies have assessed how nutrient recycling by freshwater metazoans (e.g., fish, unionid mussels) changes with a loss of species (McIntyre et al. 2007; Vaughn 2010; Vaughn et al. 2015). Although species composition (identities of the species within the community) and the functional groups represented in the community in question are important in determining community-wide nutrient recycling rates and ratios (Vanni 2002; Vaughn 2010; Benstead et al. 2010), studies have generally concluded that loss of biodiversity can have large impacts on nutrient recycling and retention in ecosystems (McIntyre et al. 2008; Vaughn

2010; Vaughn et al. 2015). All the aforementioned studies that have examined the relationship between biodiversity and nutrient recycling have been conducted in surface-associated aquatic communities, thus there is a very clear deficit on this research area in below-ground aquatic communities where nutrient recycling by consumers may play a critical role in nutrient supply to subterranean microbial biofilms. In the Edwards Aquifer, nutrient recycling by stygobionts may provide a critical ecosystem service for the microbial biofilms; spatial variation in microbial production plays a key role in the maintenance and diversity of the stygobiont communities in the Edwards Aquifer (Hutchins et al. 2016). If a substantial proportion of available inorganic nutrients in the Edwards Aquifer are supplied by the nutrient recycling of stygobiont communities, then declines in species richness in the aquifer can alter nutrient recycling and retention. However, the nature of this relationship (if any exists) has yet to be explored.

The purpose of this study was to examine the relationship between biodiversity of stygobionts and community-wide nutrient (N and P) recycling at several sites across the Edwards Aquifer. Taxon-specific nutrient excretion rates and ratios were measured in the field at three sites which differ in species composition and diversity. Field-based excretion data was subsequently linked to detailed information on the abundances of different taxa at each site in order to calculate the relative importance (contribution) of each species to the community excretion rates and ratios at each site. These data were used in probabilistic numerical simulations to examine how community-wide nutrient recycling by stygobionts is affected by the local extinction of species at each site. These data can be used to provide information on the nature of the relationship between biodiversity and ecosystem function in groundwater-based communities. This contributes

considerably to our understanding of how ecosystem function changes with loss of species from communities in aquifer systems. In addition, this material can be used to inform resource managers and policy makers as to why protection of stygobiont biodiversity in the Edwards Aquifer may be critically important for preserving of ecosystem function and the identities of species that play a critical role in those functions.

Hypothesis and Prediction 1: Stygobionts across the Edwards Aquifer will differ in their abundances and their nutrient excretion rates and ratios. I predict that body size will play a critically-important role in determining the abundances of stygobionts at sites across the Edwards. I predict that smaller-bodied taxa will be more abundant at sites, but in accordance with metabolic scaling theory (Brown et al. 2004) will exhibit higher mass-specific N and P excretion rates than larger-bodied species.

Hypothesis and Prediction 2: Variation among stygobiont species in their contribution to the community-wide nutrient recycling rate will be a function of both the relative biomass of each species in the community and their species-specific functional traits (species-specific nutrient excretion rates and ratios). I predict that species, which occupy a dominant proportion of the community biomass (biomass-dominant species), will have relatively important contributions to community-wide excretion rates. However, I also predict that species which exhibit contrasting excretion rates between individual nutrients (N or P) will likely exhibit important species identity effects in terms of their contribution to community-wide nutrient excretion.

Hypothesis and Prediction 3: The progressive loss of stygobiont species (progressive local extinction) during numerical simulations will indicate that loss of diversity will lead to changes in ecosystem functioning (e.g., nutrient recycling rates and

ratios). I predict that nutrient recycling rates will decline with declining biodiversity, but that the nature of that decline (Redundancy model, Rivet model, Linear model, etc ; Naeem et al. 2002) is unknown and likely to be dependent upon the relative biomass of species within each community and the functional traits of different species within the community.

II. MATERIALS AND METHODS

Study Sites, Field Sampling, and Estimation of Nutrient Recycling Rates

Stygobiont excretion rate data was collected in the field at three sites across the Edwards Aquifer in central Texas: Comal Springs (CS) (Comal County), Robertson Springs (RS) (Bell County), and the Artesian Well at Texas State University (AW) (Hays County). These three sites were selected because they vary in their distance from the freshwater-saline water interface in the aquifer and thus their stygobiont species richness (Hutchins et al. 2016). Sites were also chosen because they are easily accessible and have a large amount of historical sampling data for prior information. Collection of stygofaunal invertebrates at each site was done using 100 μm mesh Nitex drift-nets set for up to 24 hours. Measurement of excretion rates at each site was conducted from May - October 2016 for 12 species ($n = 2-9$ estimates per species) at AW, 7 species ($n = 1-15$ estimates per species) from CS, and 6 species ($n = 2-20$ estimates per species) from RS. Species were identified using a dissecting microscope in the field and placed in excretion chambers on-site for excretion rate measurement.

Nutrient recycling rates were determined from live invertebrates by measuring the change in dissolved N and P concentrations over a short period of time. Experiments followed a protocol similar to that used in other field-based excretion rate studies, (Vanni 2002; Benstead et al. 2010) and measured excretion rates of N and P as NH_4^+ and PO_4^{3-} , respectively. In the field, a clean and acid-washed 60 ml Nalgene container was filled with pre-filtered (Pall A/E filter) Edwards Aquifer water. Invertebrates collected in the field were gently placed in the container and then incubated in pre-filtered water for 2-4 hours. In the case of larger-bodied individuals, I placed one individual per container, but

for individuals of small-bodied taxa I placed a larger number of individuals (5-50) in a container to ensure a measureable increase in dissolved nutrients at the end of the incubation period. During incubation, samples were held at in situ aquifer temperature (23°C) and in the dark. After the incubation period, organisms were gently removed and water was filtered through a Pall A/E filter, preserved with H₂SO₄ and frozen until chemical analysis in the lab. Each sample was analyzed for NH₄⁺-N and PO₄³⁻-P using the phenate method (Solorzano 1969) and the molybdenum blue method (Wetzel and Likens, 2000), respectively. Excretion rates were calculated as the change in NH₄⁺-N or PO₄³⁻-P per individual organism per unit time (μmol NH₄⁺-N or PO₄³⁻-P/individual/h). The product of this per capita excretion rate and the relative abundance of the species was used to estimate the population-level species-specific recycling of each species. I was able to conduct $n = 71$, $n = 46$, and $n = 54$ excretion estimates for AW, CS, and RS over the course of the study, respectively (Table 1). Dry mass of individuals from each species was measured using a Mettler Toledo MX5 microscale on polystyrene weigh boats. Each organism was dried at 60 °C for 24 - 48 hours for complete dehydration of sample.

Estimation of Stygobiont Abundances

Relative abundances of the different species was determined from long-term net collections at each site. Because the precise “areal catchment” of a spring or a well site is essentially unknown, species’ abundances at each site is expressed as the number of individuals collected in a net per 24-h period. In addition, I am assuming that the organism relative abundances collected in nets at a site are reflective of the relative abundances of organisms within the aquifer. Fairly long-term and extensive records for

each site exist as a part of regular monitoring programs and I utilized the mean 24-h abundance of each species across all sampling dates at each location. Abundance data for the Artesian Well site (21 taxa collected) was based on collections made by Dr. Benjamin Schwartz (EARDC, Texas State University), Dr. Benjamin Hutchins (TPWD), and Dr. Weston Nowlin (Texas State University) which encompass a period from February 2013 to November 2015 and includes 84 sampling dates. Abundance data for Comal Springs (12 taxa) was supplied by Mr. Randy Gibson (USFWS) for a period from May 2013 to November 2015 and includes 18 sampling dates. Data for Robertson Spring (9 taxa) was supplied by Mr. Pete Diaz (USFWS) and for a period from July 2015 to August 2016, and includes 13 sampling dates. I collected excretion data on two species (*Haplotaxis gordioides* and *Almuerzothyas comalensis*) from Comal Springs which did not have previous abundance data, thus I assigned the relative abundance of the rarest species at that site.

Empirical Data Analysis

Ordinary least squares linear regression was used to describe the generalized mass-scaling of the recycling rates at each site using \log_{10} -transformed excretion rates (NH_4^+ and PO_4^{3-}) and \log_{10} -transformed organismal dry mass (mg). In some cases, it was not possible to empirically determine the excretion rates of some species because they are extremely rare and/or small. However, they appear in the historical sampling data and are representative of a portion of the community biomass. In several cases (nine taxa at AW, five taxa at CS, and three at RS), I estimated the excretion rates for species using the mass-generalized N or P equations for all species at the site to determine the excretion rates and ratios (*sensu* McIntyre et al. 2007).

In order to examine the role of species identity on nutrient (N and P) recycling rates, I examined the contribution of each species to community-wide (aggregate) nutrient recycling based on mass-generalized (based solely on body size) recycling rates for each species and compared this to empirically-determined species-specific recycling rates. Mass-generalized rates were derived from the mass-scaling relationships of N and P recycling across all species, thereby excluding species-specific influences such as growth rates, nutrient content of body tissues, or diet (McIntyre et al. 2008). The difference between these species-specific and generalized estimates represents the influence of species identity. I also evaluated the species-specific influence on the recycled N:P ratio by determining the difference in aggregate N:P recycling between communities including and lacking each species.

Extinction Simulations Using Nutrient Excretion Estimates

Local extinctions from a community are typically not random and are in response to stressors that exploit a species' sensitivity until populations disappear (Duffy 2003). Using an approach first utilized by McIntyre et al. (2007), I evaluated the ecosystem functioning consequences of the loss of various macroinvertebrate stygobiont species at each of the three Edwards Aquifer sites. I evaluated how community wide N and P excretion rates and the aggregate N:P excreted by the community changes with sequential species losses across a variety of extinction scenarios. The scenarios that were simulated are (1) random and progressive species extinction with no compensation as species were lost, (2) random and progressive species extinction with energetic compensation to the remaining species in the community after a species was lost, (3) ordered and sequential extinctions from the community in order of body size, best and worst case scenarios,

trophic position, and relative abundance in the community. Ordered extinctions were conducted with probabilities of extinction for each species being directly or inversely proportional to specific traits. An $n = 500$ simulations was performed at every level of species richness for each scenario (Fig. 8). These simulations were written and implemented in R (R Core Team 2015).

A more detailed explanation of the various extinction scenarios is below:

1. *Random Extinctions*

- a. *No Compensation* - Completely random extinction of species with no compensatory increase in populations of the remaining trophically-similar species. All species in these models have an equal probability of going extinct at any point during the simulation.
- b. *Energetic Compensation* - After a random extinction of a species, there is a compensatory population growth of the remaining species in the community. This compensatory energy/growth was allocated to species in the same feeding guild as the extinct species. The energy lost by an extinction was used to generate more individuals for the surviving guild members in proportion to their relative energy usage, effectively preserving both total energy flow and energetic partitioning. Feeding guilds were determined using existing data and publications (Hutchins et al. 2016; Koehl and Strickier 1981; Holsinger 1980; Reddy 2011; Cannon 1926; Holsinger 1973; Dahl 1978; Swan and Palmer 2006; Bruce and Short 1993; Fryer 1964). Metabolic scaling theory was used to estimate compensatory responses, which dictates that population density and body

size are a function of a species energy usage (metabolic rate: $\sim \text{Mass}^{3/4}$) (Brown et al. 2004).

2. *Ordered Extinctions*

- c. *Relative Abundance* - The extinction of the species with the smallest population has the highest probability because they are typically the most sensitive to ecological stresses (Solan 2004; Duffy 2003). This results in an estimate of how the loss of “rare” species affects the nutrient recycling. Stygobiont abundance was ascertained using historic data and sampling.
- d. *Body size* - There is a general negative relationship between susceptibility to local extinction and body size; larger-bodied species tend to be lost from communities first (Solan 2004; McIntyre et al. 2007). In this scenario, species with higher body mass (dry mass in mg) are simulated to have a larger probability of extinction. Dry mass of the various stygobiont species was ascertained as described above.
- e. *Best and Worst Case Scenarios* - Species with the smallest contribution to recycling rates have a higher probability of extinction for the “best case” scenario and a lower probability for the “worst case” scenario. The resulting model portrays conditions in which the best and worst possible outcomes of stygobiont extinctions on nutrient recycling by the community is shown.
- f. *Trophic position* - Simulations were carried out by having the higher trophic level species becoming extinct first. Again, higher trophic level species are often more sensitive to ecological perturbation and are often

more prone to local extinction (Covich et al. 1999; Duffy 2003). Trophic position was derived from stable isotope data in the published literature as well as other sources (e.g., Hutchins 2016). However, some species have not had their trophic position evaluated, and in those cases, the average trophic position of similar species, based upon literature-defined feeding guild and diet, was used.

III. RESULTS

Extinction Scenarios

The order in which species are lost and the capacity of surviving species to compensate strongly affected the outcome of subterranean invertebrate extinctions on nutrient recycling. Random loss of species richness resulted in a linear decline of both N and P recycling for all three sites (Fig. 1-3 A and H). When compared across sites, the predictability of excreted N:P ratio was inversely related to species richness (Fig. 1-3 O-U) and was most constrained in RS due to the narrow range of N:P recycling ratios across species (molar N:P ranged from 5.8 to 9.7). At the AW site, in the absence of compensatory effects there was a strong bifurcation in both the N and P recycling of AW due to *Palaemonetes antrorum* contribution of 94% and 95% of total recycled N and P in the community, respectively. This is due to *P. antrorum*'s high abundance (103 individuals/24 hours) and high individual biomass/size (16.3 mg DM average per individual). A clear bifurcation is present at CS for both N and P recycling, reflecting scenarios where *Stygobromus sp.* was lost or retained in the community; *Stygobromus sp.* contributed 72% to the N and P community-wide recycling rate at CS. For the recycling of P in CS community *Lirceolus sp.* contributed an additional 14% to the community-wide rates and its extinction led to another large break in P recycling. Due to a low biodiversity as well as similar contributions to recycling amongst species at RS, loss of species caused a decline in ecosystem functioning that is relatively unpredictable with no obvious bifurcations when compared to the other sites.

When energetic compensation is allocated to populations of surviving species in the form of increased abundance during random extinction scenarios, then

overall community-wide recycling rates have the opportunity to actually increase (Fig. 1-3 B and I). In fact, AW community-level recycling rates can increase up to 600% total N and 140% total P when much smaller species (0.15 mg average for remaining species) from the same guild energetically compensate for loss of *P. antrorum* due to their higher mass-specific excretion rates. This is due to *P. antrorum*'s high abundance (103 individuals/24 hours) and large body size, resulting in a large amount of biomass and energy being transferred to other species within the scraper/benthic forager guild. At the CS and RS sites, increases in community-wide recycling rates with compensatory increases was much less pronounced due to both sites not containing a large-bodied consumer which was also numerically and biomass dominant in the community. CS and RS however, both display a dependence on recycling by the presence of the predator guild due to that guild of consumer contributing a large amount to nutrient recycling (75%-85% of the community recycling rate). At the CS and RS sites, major loss of ecosystem functioning was driven by loss of entire trophic guilds instead of a small number of species due to the loss of compensatory energy flow.

Roles of Species to Community-Wide Nutrient Recycling

Ordered extinction simulations produced differing outcomes, dependent upon the particular scenario involved. Ordered extinctions by trophic position generally resulted in a scenario equivalent to random extinctions with no compensation (Fig. 1-3 D, K, and R). Best case extinction scenarios were associated with the loss of rare species representing relatively weak effects on ecosystem functioning due to the relatively small amount of total biomass rare species contribute. The ordered extinction of larger body size taxa

resulted in scenarios like worst-case scenarios, exemplifying the strong relationship between nutrient recycling rates and the body size of taxa.

The alternative extinction scenarios above resulted in widely different patterns, reflecting a considerable skew in the proportional contributions of individual species to aggregate nutrient recycling at each site. The estimated contributions of different species to community-wide nutrient recycling rates were dominated by the top three species at each site, which accounted for 90-96% of the total N and P excretion at all three sites (Fig. 4 A-C). As a result, only *P. antrorum* in AW affected the aggregate recycled N:P ratio by more than ± 1.0 , whereas the largest effect at CS and RS were due to *Lirceolus* sp. (-0.4) and *Stygobromus russeli* (+0.22), respectively (Fig. 4 D-F). However, there was a relatively large range of recycling ratios exhibited by individual species (molar N:P ratios of excreted nutrients among species ranged from 6.9 - 43.9).

Effects of Species Identity on Nutrient Recycling

Species identity and the relative biomass within the community explained a large amount of the variation among species in contributions to community-wide nutrient recycling. Across all species, biomass explained 80% of variation in contributions to N recycling (Fig. 6 A-C) in AW ($F_{1,69} = 261.22$, $P < 0.001$), 65% in CS ($F_{1,44} = 82.5$, $P < 0.001$), and 82% in RS ($F_{1,52} = 233.04$, $P < 0.001$). In addition, biomass explained similar amounts of variation in P recycling (Fig. 7 A-C) in AW (86%, $F_{1,67} = 406.21$, $P < 0.001$), CS (59%, $F_{1,44} = 62.54$, $P < 0.001$), and in RS (83%, $F_{1,52} = 258.47$, $P < 0.001$). In order to further discern the effect of species identity, I compared species' contributions to aggregate nutrient recycling derived from species specific versus mass generalized excretion rates. Species-specific and generalized estimates of contributions to

total N and P recycling were similar and concordant at the CS site; however, in RS and AW the magnitude between species-specific and mass-generalized rates differed for both N and P (Fig. 5). A fairly striking difference between species specific (50.7 $\mu\text{mol/h}$) and mass-generalized N excretion rates (89.9 $\mu\text{mol/h}$) was evident for *P. antrorum*, indicating that the contribution of *P. antrorum* to community-wide nutrient recycling was lower than predicted solely by its biomass in the community. In contrast, *Lirceoulus sp.* at RS site had substantially lower mass-generalized N and P recycling rates than the species-specific rates, indicating that species identity can result in a much greater contribution to community-wide nutrient recycling than would be predicted solely from its biomass in the community. These data also indicate that the relative importance of each species to the community-wide recycling of N or P differs depending upon the specific nutrient in question. For example, *Texiweckeliopsis insolita* at the AW site was the third highest contributor to N recycling at the site, but was not among the top contributors in terms of P recycling (Fig. 5 A and D).

IV. DISCUSSION

This study provides a quantitative assessment of how loss of stygobiont species richness may affect ecosystem functioning. My results indicate that a decline in species richness, random or nonrandom, is highly likely to alter nutrient recycling in various ways as observed in previous studies with other taxa (Solan 2004; Balvanera et al. 2006; McIntyre et al. 2007). As predicted, complex community-level effects of extinctions were found when I compared the various extinction scenarios among the study sites as well as in comparison among the two nutrients (N or P). Nutrient recycling, as an ecosystem process, is positively related to the biomass of organisms in the community (i.e., a biomass-dependent process at the individual and community levels) (Allen and Gillooly 2009; Vanni 2002). Therefore, in scenarios where loss of rare species occurred, relatively minor effects on nutrient recycling were observed due to low population-level biomass of uncommon species. In contrast, extinction scenarios ordered by large size led to relatively large community-wide nutrient recycling effects due to the biomass-dependent nature of the ecosystem function. Trophic position scenarios indicate intermediate effects similar to random extinctions without energetic compensation, reflecting the relatively small range of trophic levels, as well as, higher contributions amongst species with intermediate trophic levels.

Energetic compensation of species after extinctions resulted in situations in which a ranging from large increases to small decreases in ecosystem functioning until a substantial number of species from the same feeding guild were lost. The resulting patterns initially appear to suggest a decoupling of biodiversity and ecosystem functioning, but actually reflect the directionality of ecosystem functioning responses are

dependent upon the functional diversity of the community and the order in which species are lost. In extinction scenarios, as species were progressively lost from the community, the loss of entire functional groups of organisms became more likely (Balvanera et al. 2006). Thus, stygobiont taxonomic diversity provides a buffer for ecosystem processes by providing functionally redundant species that are available to compensate after extinctions (Naeem et al. 2002; Hooper et al. 2005). The degree to which the amount of compensation occurs depends upon the diversity within each functional group.

In the present study, I also observed that the range in ecosystem functioning responses when compensation was allowed were somewhat variable, ranging from large increases to minor decreases. For example, at the AW site, the loss of *P. antrorum* from the community led to a suite of responses, including substantial increases in nutrient recycling rates by the community. In this scenario, the loss of the large biomass of *P. antrorum* from the community coupled with compensatory increases in the biomass of smaller bodied taxa with higher mass-specific nutrient recycling rates lead to an increase in community-wide nutrient recycling. Despite the findings of this study and others which have examined the ecosystem functioning effects of compensatory responses of members of the community when species extinctions occur (McIntyre et al. 2007), the precise effects of community reorganization after extinctions in complex and/or speciose communities is unfortunately not well understood and requires further exploration.

Similar to previous studies which have examined the contribution of individual species to ecosystem processes in natural ecosystems (Solan 2004; Balvanera et al. 2006; McIntyre et al. 2007), the present study found that a few species dominated community-level nutrient recycling (Fig. 4). The species which played these key roles typically

occupied a large portion of the community biomass, reflecting the importance of biomass to ecosystem functioning. However, species-specific and mass-generalized excretion rates differed for a variety of species in the community in both magnitude and rank order (Fig. 5). These findings indicate that the relative importance of species in the community on nutrient recycling is not only impacted by biomass, but also by species-identity related factors, such as body stoichiometry, dietary nutrient content, and growth rate (; Vanni et al. 2002; Sterner and Elser 2002). Thus, species-specific characteristics can have substantial effects on ecosystem functioning which would not be predicted by the relative biomass of species within the community. However, I also found that relative biomass alone accounted for ~80% of the variation in nutrient recycling rates at the AW and RS sites, but accounted for ~60% at the CS site. This result suggests that the degree to which species-specific ecological and physiological factors can influence community-wide nutrient recycling can vary between sites and communities, depending upon the diversity and composition of communities.

On a global scale, freshwater aquifers are subject to increasing anthropogenic influences such as introduction of pollutants, groundwater extraction, and elimination or reduction of recharge (Boulton et al. 2008). Alteration and degradation of groundwater habitats has the potential to cause local extinction of stygobiont species. In the study presented here, I show that stygobionts can play an important role in nutrient recycling in subterranean aquatic ecosystems and their extinction can have substantial consequences for subterranean communities. I also demonstrated a quantitative approach for assessing the impact of stygobiont extinctions on ecosystem functioning and found that as stygobiont species richness declined, the effects of extinction were dependent upon the

order of extinction and compensatory responses of surviving species. In addition, I found that the relative biomass of each species in the community as well as species-specific functional traits influenced the nutrient recycling of stygobiont species. Future studies should evaluate if stygobionts provide enough total ecosystem functioning, in the form of excreted N and P, to support or at least aid in situ chemosynthetic bacterial production (Sarbu et al. 1996; Roach et al. 2011; Hutchins et al. 2016).

Table 1. Stygobiont taxa collected from AW, CS, and RS between May 2016-October 2016. Per capita excretion rates are reported as $\mu\text{mol NH}_4^+\text{-N}$ or $\text{PO}_4^{3-}\text{-P}$ /individual/h. Abundance numbers are reported as the mean number of organisms caught standardized for a 24-h collection period effort. Dry mass of organisms is reported in mg. Feeding guild and trophic position data were derived from published literature as well as other sources. Asterisks indicate per capita excretion rates are based upon mass generalized N and P equations.

Artesian Well							
Taxa	Per capita N	Per capita P	N:P of Species	Mass (mg)	Abundance(Ind/24)	Trophic level	Feeding guild
<i>Stygobromus flagellates</i>	0.1968	0.0195	10.07	2.825	2.84	4.14	Predator
<i>Stygobromus longipes</i>	0.2572	0.0184	14.01	1.211	0.05	3.6	Predator
<i>Stygobromus russeli</i>	0.2031	0.0133	15.31	0.648	0.06	1.27	Predator
<i>Seborgia relicta</i>	0.0156	0.0022	7.23	0.050	3.65	2.68	Scraper/Benthic forager
<i>Texiweckelia texensis</i>	0.1092	0.0130	8.42	0.362	0.95	2.29	Filter feeder
<i>Texiweckelopsis insolita</i>	0.1617	0.0081	19.87	0.288	3.47	3.71	Scraper/Benthic forager
<i>Lirceolus sp.</i>	0.0784	0.0094	8.35	0.445	2.75	3.52	Scraper/Benthic forager
<i>Cirolanides texensis</i>	0.4067	0.0517	7.87	9.963	1.61	3.44	Predator
<i>Phreatodrobia nugax</i>	0.0146	0.0020	7.41	0.060	2.39	2.31	Scraper/Benthic forager
<i>Ostracods sp.</i>	0.0043	0.0006	7.38	0.009	65.25	2.64	Filter feeder
<i>Copepods sp.</i>	0.0051	0.0006	7.89	0.010	93.11	2.64	Filter feeder
<i>Palaemonetes Antrorum</i>	0.4924	0.0711	6.93	16.312	103.03	3.22	Scraper/Benthic forager
<i>Calathaemon holthuisi*</i>	0.8787	0.0860	10.22	16.312	0.04	2.95	Filter feeder
<i>Tethysbaena texana*</i>	0.0226	0.0024	9.54	0.0767	0.18	2.68	Scraper/Benthic forager
<i>Artesia subterranea*</i>	0.0891	0.0091	9.79	0.5715	0.17	4.28	Predator
<i>Phreatodrobia rotunda*</i>	0.0238	0.0025	9.55	0.0828	0.02	2.31	Scraper/Benthic forager
<i>Phreatodrobia plana*</i>	0.0191	0.0020	9.51	0.0600	1.70	2.31	Scraper/Benthic forager
<i>Allotexiweckelia hirsuta*</i>	0.1241	0.0126	9.85	0.9286	0.12	3.72	Predator
<i>Holsingerius samacos*</i>	0.0948	0.0097	9.80	0.6259	0.13	2.69	Filter feeder
<i>Parabogidiella americana*</i>	0.0520	0.0054	9.69	0.2595	0.18	3.6	Predator
<i>Haideoporus texanus*</i>	0.0828	0.0085	9.78	0.5135	0.09	2.95	Predator

Comal Springs							
Taxa	Per capita N	Per capita P	N:P of Species	Mass (mg)	Abundance(Ind/24)	Trophic level	Feeding guild
<i>Stygobromus pecki</i>	0.2947	0.0176	16.70	2.413	4.44	4.01	Predator
<i>Stygobromus sp.</i>	0.1302	0.0124	10.49	0.635	91.11	3.6	Predator
<i>Mexiweckelia hardeni</i>	0.0558	0.0065	8.57	0.462	7.55	2.17	Scraper/Benthic forager
<i>Seborgia relicta</i>	0.0341	0.0008	43.97	0.068	7.66	2.68	Scraper/Benthic forager
<i>Lirceolus sp.</i>	0.1078	0.0132	8.16	0.622	17.61	1.91	Scraper/Benthic forager
<i>Haplotaxis Gordioides</i>	0.1195	0.0158	7.55	1.053	0.11	2.68	Scraper/Benthic forager
<i>Almuerzothyas comalensis</i>	0.2171	0.0228	9.53	1.349	0.11	3.6	Predator
<i>Ingolfiella sp.*</i>	0.0134	0.0012	11.09	0.042	0.11	2.68	Scraper/Benthic forager
<i>Comaldessus stygius*</i>	0.0887	0.0085	10.44	0.513	0.77	2.78	Predator
<i>Texanobathynella bowmani*</i>	0.0334	0.0031	10.77	0.141	0.11	2.64	Filter feeder
<i>Artesia subterranea*</i>	0.0962	0.0092	10.42	0.571	0.50	4.28	Predator
<i>Cirolanides texensis*</i>	0.8356	0.0859	9.73	9.963	0.77	5.1	Predator

Robertson Springs							
Taxa	Per capita N	Per capita P	N:P of Species	Mass (mg)	Abundance(Ind/24)	Trophic level	Feeding guild
<i>Stygobromus russeli</i>	0.0629	0.0065	9.73	0.8781	5.16	3.60	Predator
<i>Stygobromus bifurcatus</i>	0.1552	0.0164	9.44	3.2618	2.58	3.60	Predator
<i>Lirceolus sp.</i>	0.0438	0.0047	9.35	0.2684	2.98	2.68	Scraper/Benthic Forager
<i>Caecidotea reddelli</i>	0.1367	0.0185	7.37	2.6319	0.15	2.68	Scraper/Benthic Forager
<i>Phreatoceras taylori</i>	0.0052	0.0009	5.87	0.0486	1.33	2.68	Scraper/Benthic Forager
<i>Phreatodrobia sp.</i>	0.0126	0.0017	7.24	0.0582	3.92	2.68	Scraper/Benthic Forager
<i>Phreatodrobia micra*</i>	0.0084	0.0010	8.38	0.0600	0.48	2.68	Scraper/Benthic Forager
<i>Ostracods sp.*</i>	0.0021	0.0003	8.10	0.0094	1.18	2.64	Filter feeder
<i>Blind Dytiscidae*</i>	0.0418	0.0048	8.72	0.5135	0.06	3.60	Predator

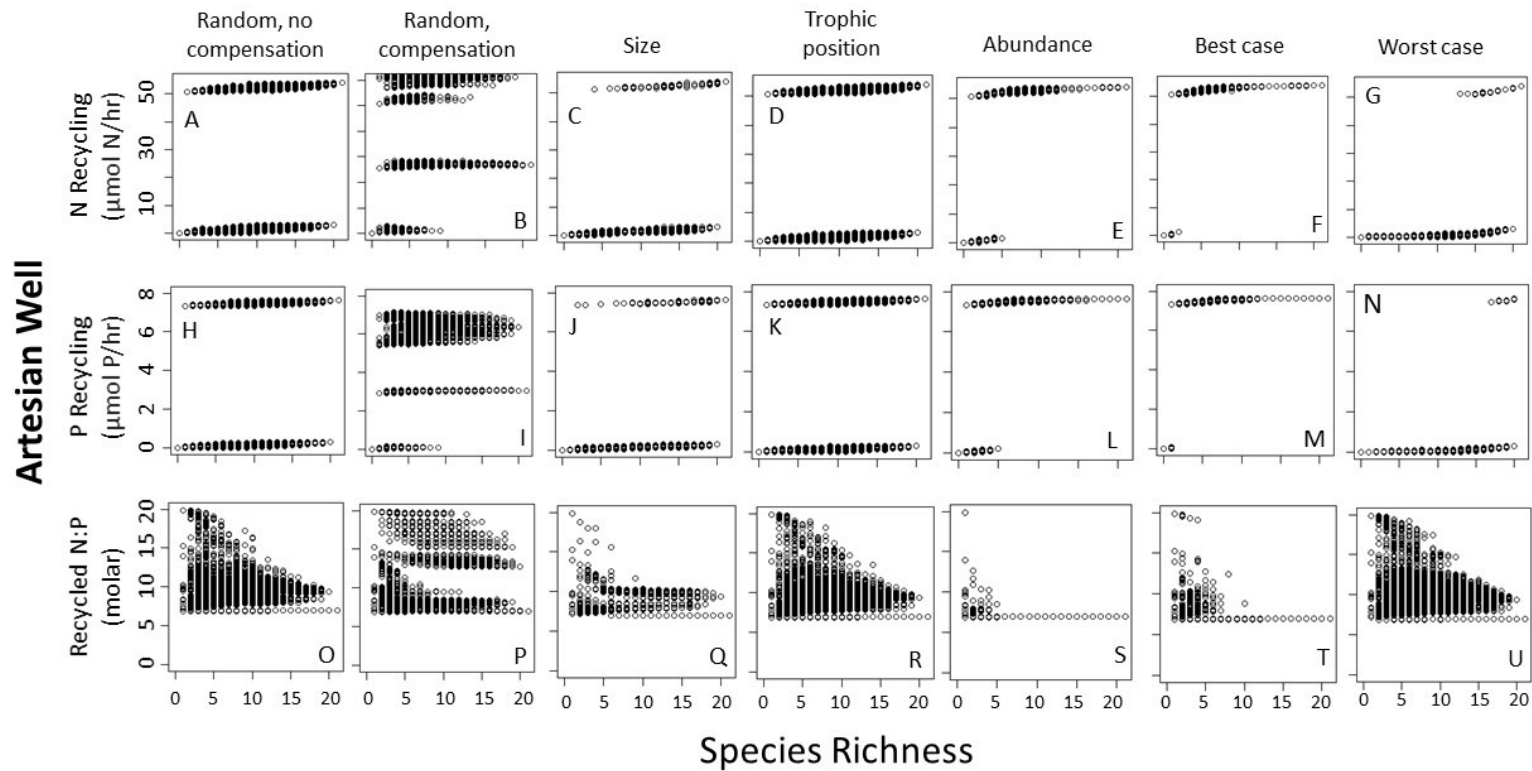


Figure 1. Effects of stygobiont species extinctions on N (A-G) and P (H-N) nutrient recycling, as well as their ratio (O-U), in AW. The simulations shown are of random extinctions without (A, H, and O) or with (B, I, and P) compensation by surviving species, and ordered extinctions illustrating increasing risk with large body size (C, J, and Q), high trophic position (D, K, and R), and low abundance (E, L, and S). For comparison, best (F, M, and T) and worst case (G, N, and U) outcomes of extinctions on nutrient recycling are shown. Figures B and I have a maximum Y value of 120 and 20 respectively, to account for increases in ecosystem functioning due to compensation. Note the bifurcating patterns present across N and P simulations caused by *P. antorum*'s dominance in nutrient recycling.

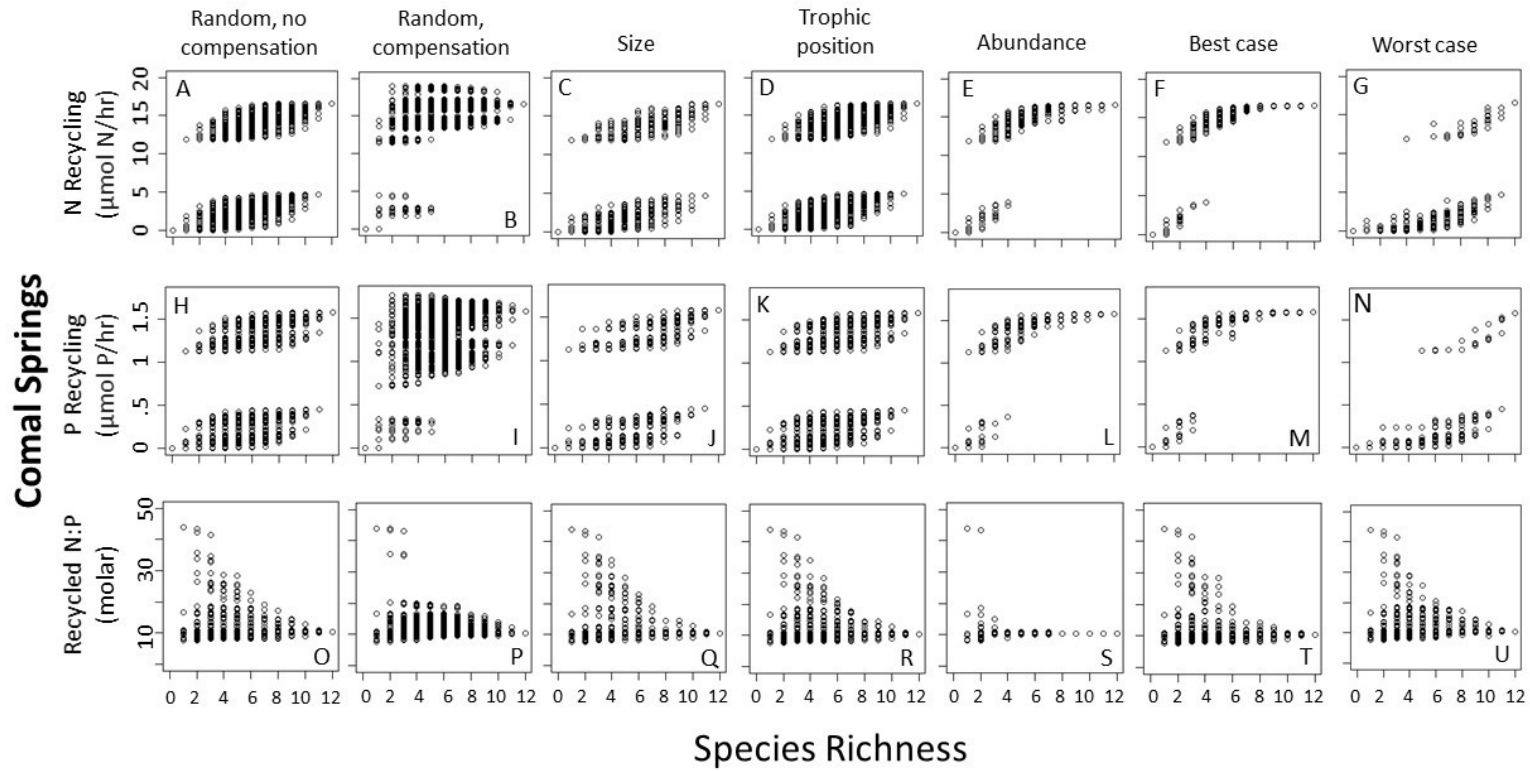


Figure 2. Effects of stygobiont species extinctions on N (A-G) and P (H-N) nutrient recycling, as well as their ratio (O-U), in CS. The simulations shown are of random extinctions without (A, H, and O) or with (B, I, and P) compensation by surviving species, and ordered extinctions illustrating increasing risk with large body size (C, J, and Q), high trophic position (D, K, and R), and low abundance (E, L, and S). For comparison, best (F, M, and T) and worst case (G, N, and U) outcomes of extinctions on nutrient recycling are shown. Note the bifurcating patterns present across N and P simulations caused by *Stygobromus sp.* dominance in nutrient recycling as well as slight interactions of *Lirceolus sp.* evident in P recycling.

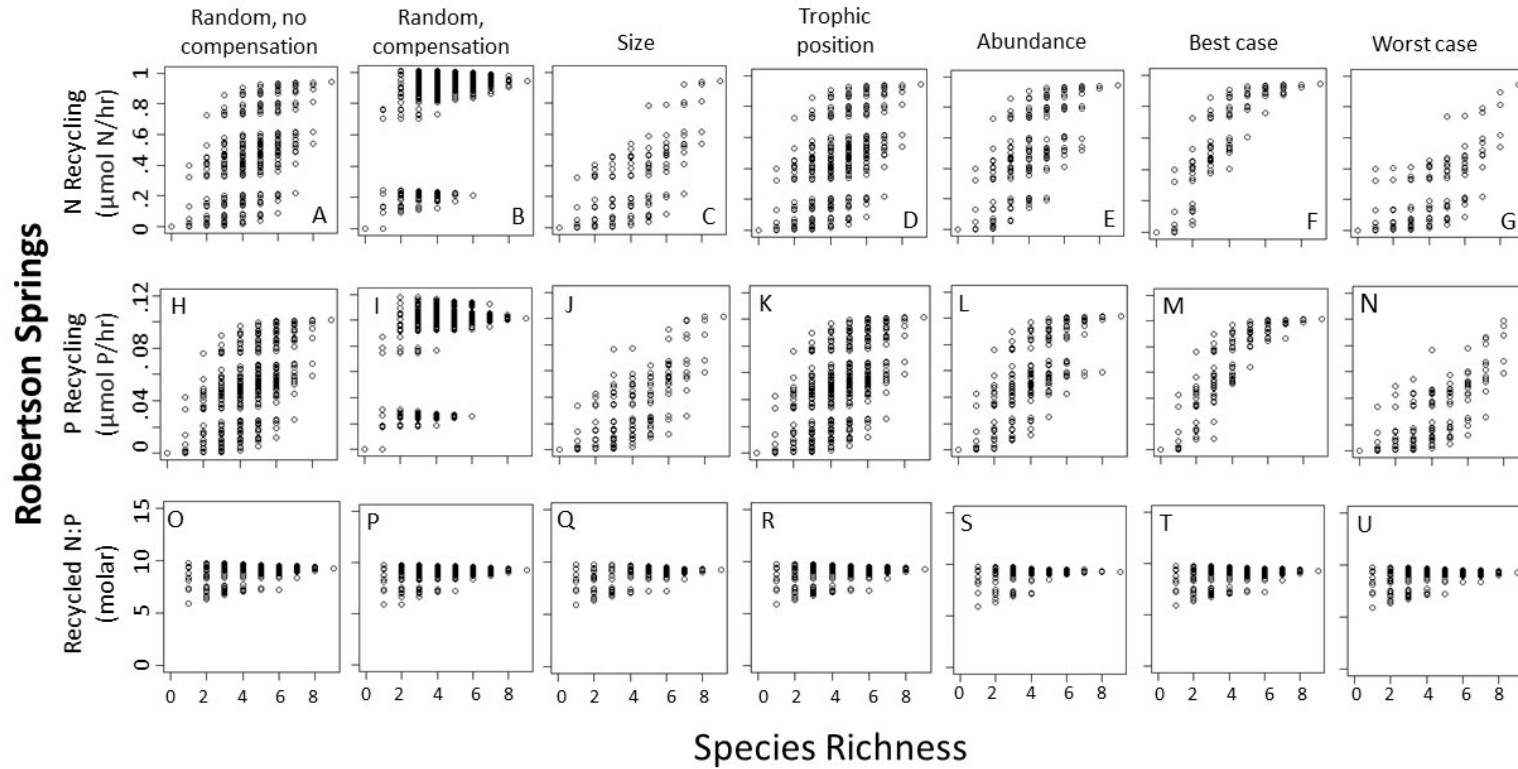


Figure 3. Effects of stygobiont species extinctions on N (A-G) and P (H-N) nutrient recycling, as well as their ratio (O-U), in RS. The simulations shown are of random extinctions without (A, H, and O) or with (B, I, and P) compensation by surviving species, and ordered extinctions illustrating increasing risk with large body size (C, J, and Q), high trophic position (D, K, and R), and low abundance (E, L, and S). For comparison, best (F, M, and T) and worst case (G, N, and U) outcomes of extinctions on nutrient recycling are shown. Note the weak trifurcating patterns present across N and P simulations associated with *S. bifurcatis*, *S. russeli*, and *Lirceoulus sp.* dominance in nutrient recycling.

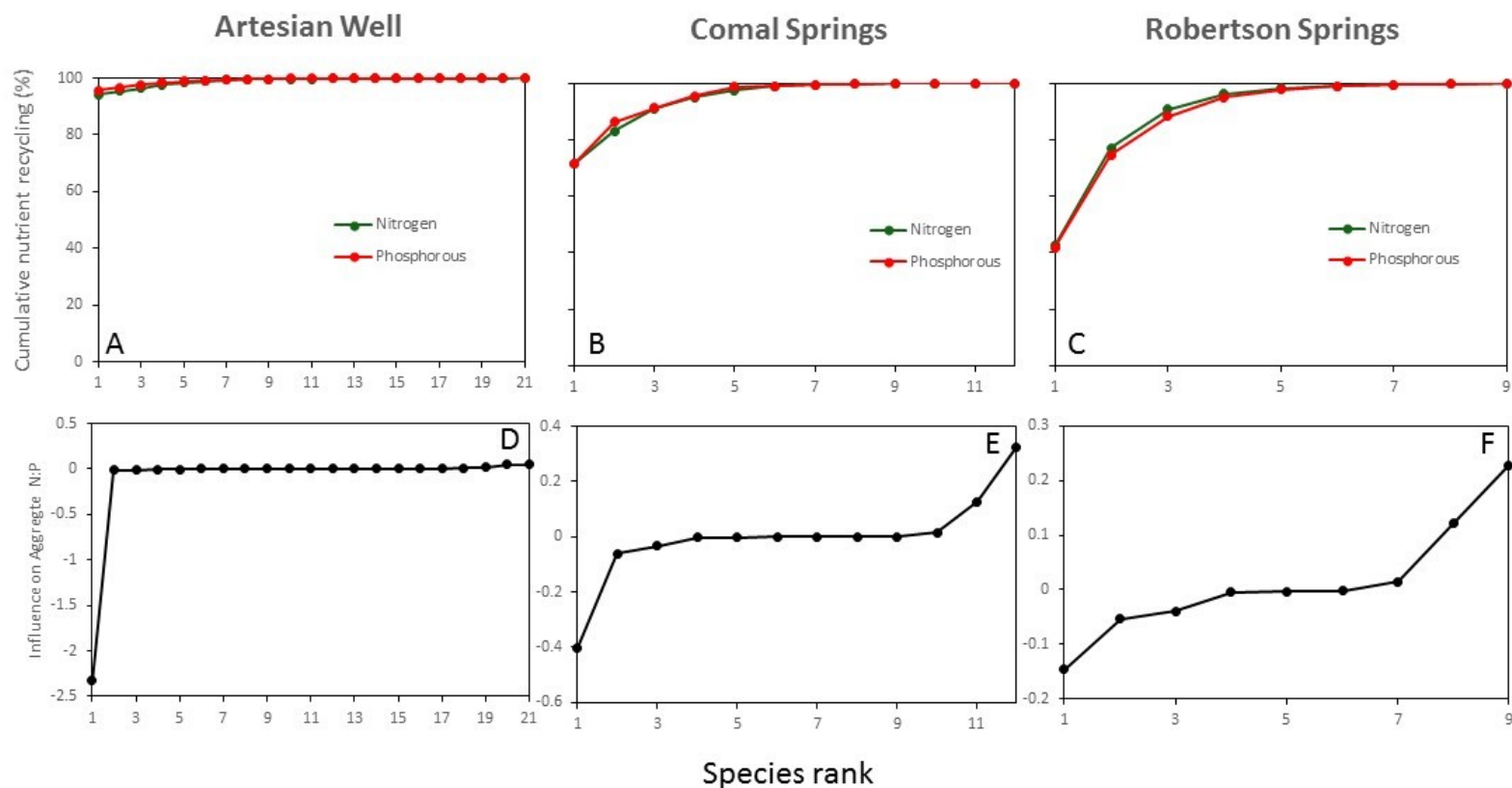


Figure 4. Contributions of individual stygobiont species to aggregate nutrient recycling in AW, CS, and RS. Images depict species in rank order of contributions to N and P recycling (A, B, and C) and influence on aggregate N:P (D, E, and F). Note the order of particular species differs between the species rank of cumulative nutrient recycling (N and P) and influence on excreted N:P.

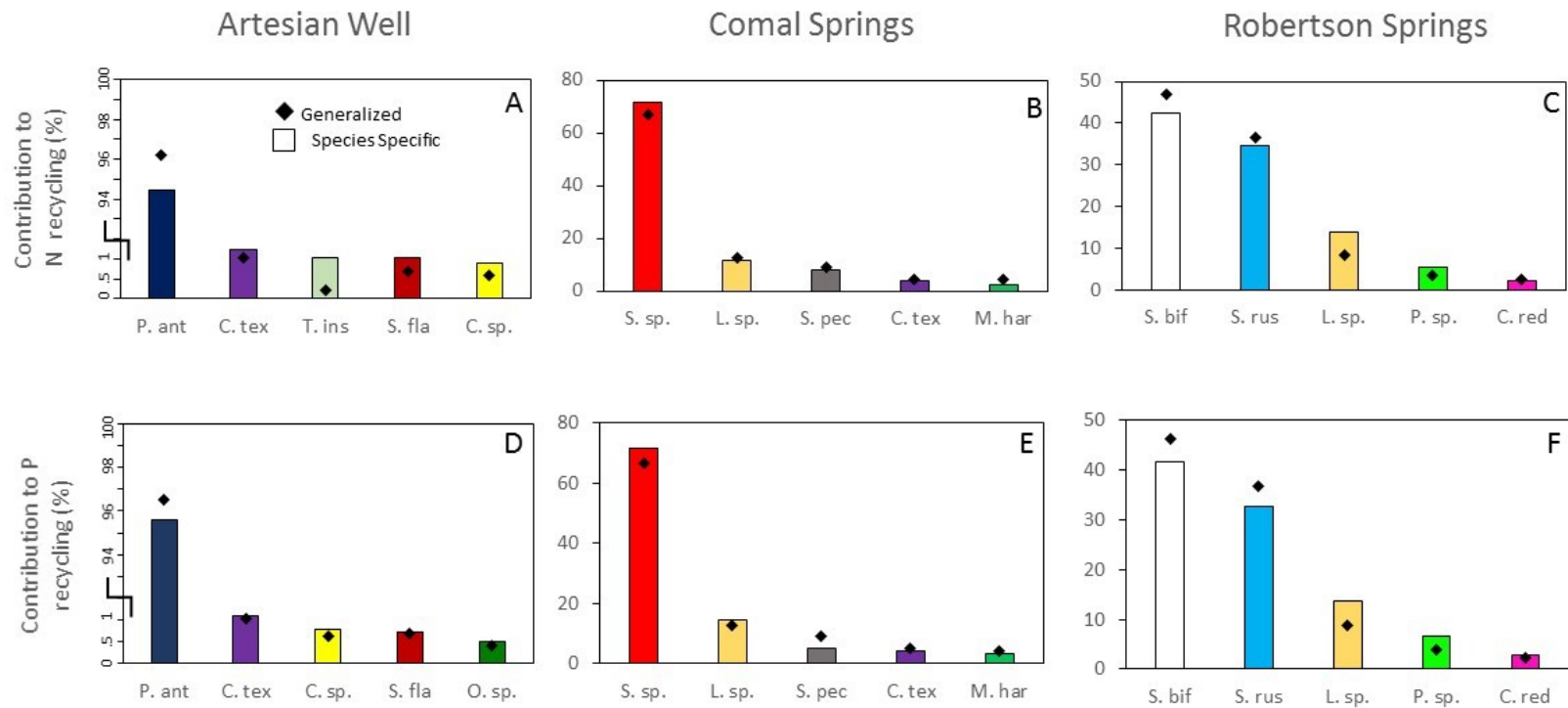


Figure 5. Identity of the top five species contributing most to community-level N (A-C) and P (D-F) recycling in AW, CS, and RS. Colors indicate individual species and are labeled with taxonomic abbreviations that carry across sites. Note the difference in rank order of species contributing to N and P recycling in AW. Species identity influences are portrayed by the difference between mass generalized (diamonds) and species specific (bars) recycling rates. There is a break in graphs A and D between 1.5% and 92% to accommodate for *P. antrorum*'s dominance in nutrient recycling.

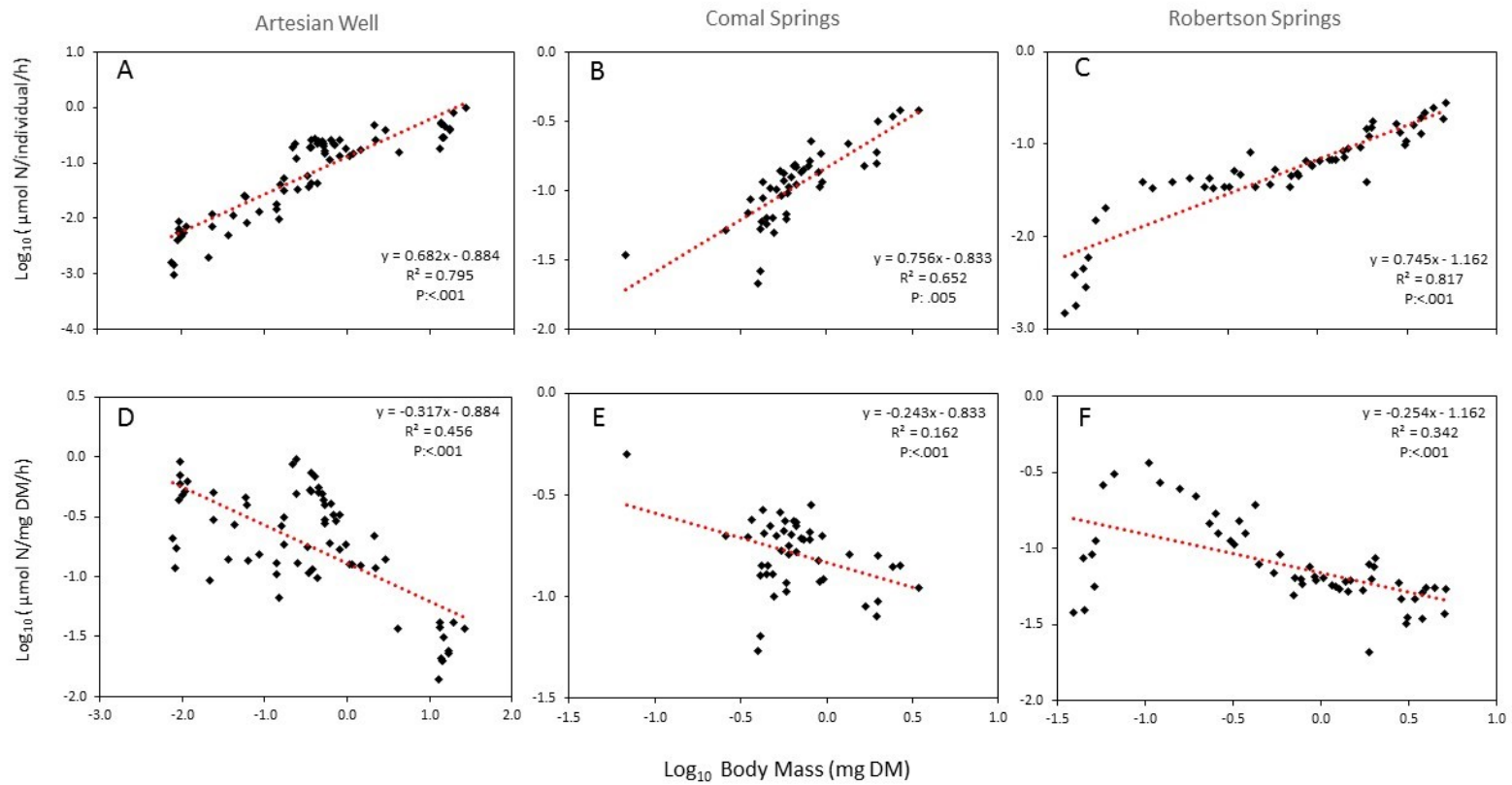


Figure 6. Relationship between N excretion rates and organism body mass for stygobiont species (mg DM) in AW, CS, and RS. Images depict ordinary least squares linear regressions between per capita N excretion rate (A-C) and mass-specific N excretion rate (D-F) against organism body mass for stygobiont species (mg DM). Regression equations, r^2 values, and p -values are shown for each regression.

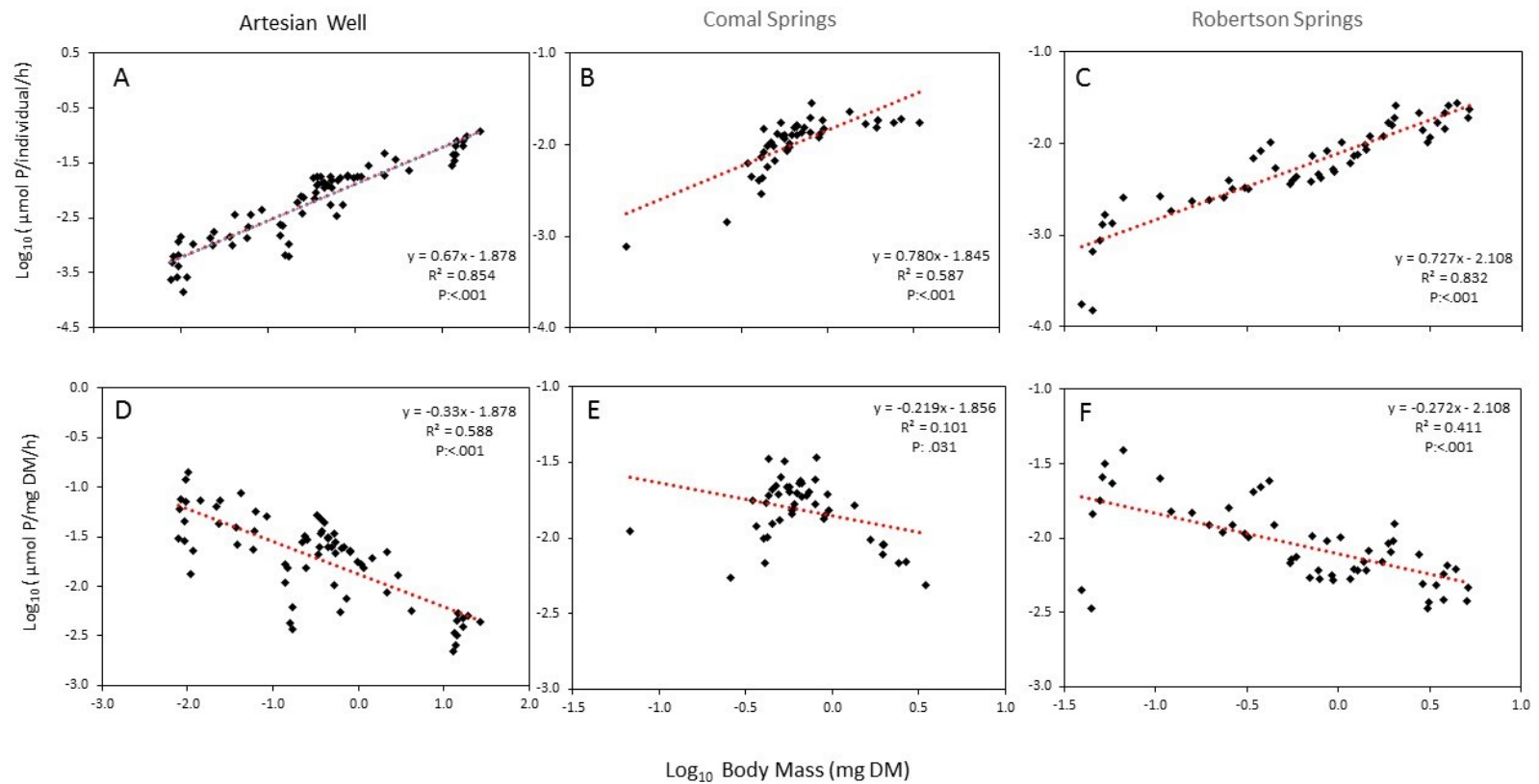


Figure 7. Relationship between P excretion rates and organism body mass for stygobiont species (mg DM) in AW, CS, and RS. Images depict ordinary least squares linear regression between per capita P excretion rate (A-C) and mass-specific P excretion rate (D-F) against organism body mass for stygobiont species (mg DM). Regression equations, R^2 values, and p-values are shown for each regression.

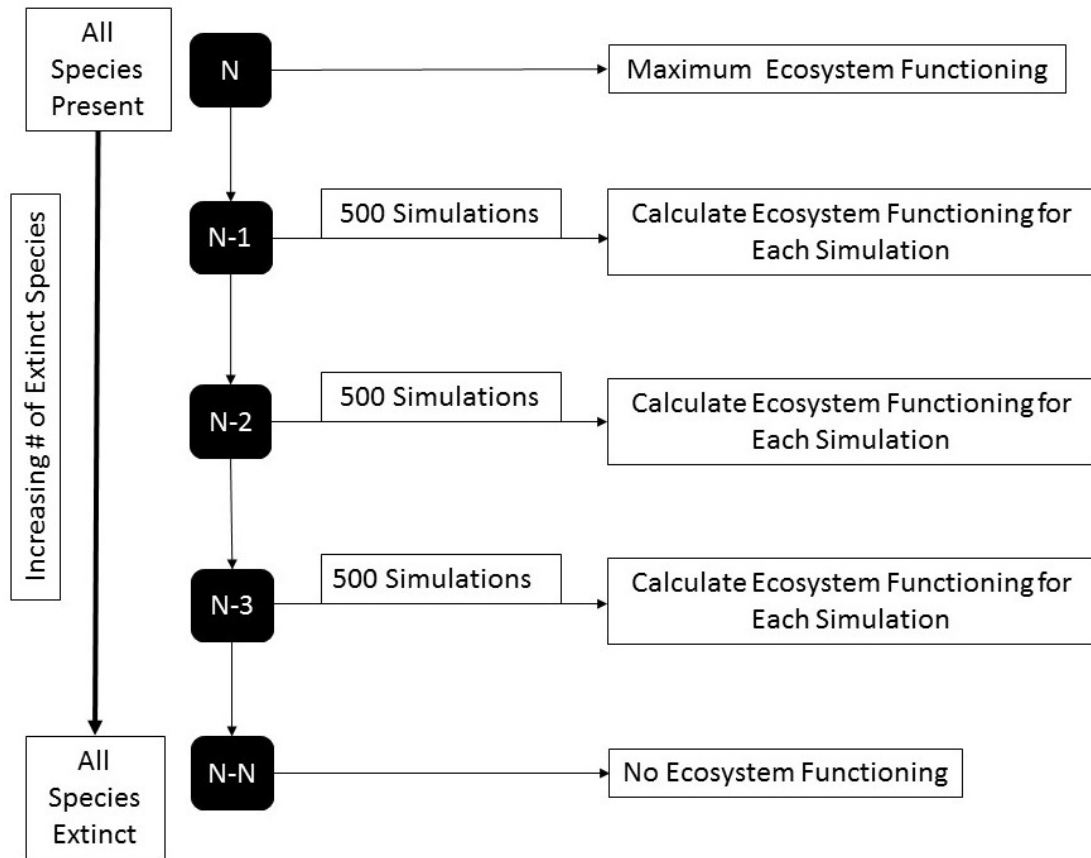


Figure 8. Conceptual model of extinction simulations. N is the total number of species within a model and represents maximum ecosystem functioning. Ecosystem functioning is calculated for each of the 500 simulations that occur at each species richness level until all species have gone extinct. N-N is representative of all species being simulated to extinction, thus the ecosystem functioning contributed by these species has ceased.

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