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Ecole Doctorale Sciences de la Nature
et de l'Homme : Evolution et Ecologie

Lausanne, October 2nd, 2023

Object: Report on the manuscript of doctoral thesis of S. Charberet

S. Charberet submitted a relatively short 107 page-manuscript entitled "Animal-mediated terrestrial nutrient dynamics: investigating wastes, trophic transfer, and isotopic patterns" as a doctoral thesis. The document comprises a very concise introduction, three chapters structured as scientific articles and a final synthesis. The manuscript is written in English, with a high quality in the writing language.

The overall scientific framework of the doctoral work lies within the overlooked role of animals within terrestrial nutrient-dynamics, within the line of the concept of zoogeochemistry as formalized, at least for carbon, by Schmitz et al., 2018 [10.1126/science.aar3213](https://doi.org/10.1126/science.aar3213). Herein, the candidate focuses on the more specific role of animals' waste in modulating nutrient cycling.

I started the reading of the thesis with a lot of enthusiasm. While the topic is not as new in aquatic science as it is for terrestrial ecology, it is pretty fascinating, and the overall question is a gold mine for a Ph.D. thesis. Indeed, the first two paragraphs of the introduction act as quite a teaser. But after that, the introduction fails its typical missions for a Ph.D. thesis, i.e., to provide the minimal concepts that are necessary to understand the stakes and hypotheses of the work, to give the state of the art of the question at stake (what we know and do not know yet) from which a set of knowledge gaps are identified. Finally, we expect the thesis introduction to raise several sub-questions or hypotheses that will at least partly address the identified knowledge gaps.

Herein, unfortunately, we miss the conceptualization and state of art. The role of wastes in soil nutrient cycles relies on the fact that digestion repackages nutrients. This is especially true for herbivores, whose wastes supposedly have a nutrient stoichiometry more favorable for nutrient accessibility or mineralization than the raw plant material animals have fed on. The stoichiometric theory is then central to the thesis. At minima, the reader needs an introductory presentation on the fundamentals of the concept. We expect related material from Sterner and Elser 2002, Zechmeister-Boltenstern et al., 2015 (which comes only at the very end), and inputs from the overall conceptualization paper by Sitters et al., 2017.

Similarly, the metabolic theory in Ecology is central to the topic and it would be necessary to provide the corpus of theories on which the work is based. There, we would expect the figure on stoichiometry versus body size from Elser 2007. In the current introduction format, it is hard to assess whether the concepts have been adequately understood or if the candidate remained at the very surface.

Similarly, the state of art is very vague, giving the impression of a shallow understanding. Several key articles, high-profile for most, require more than a single, superficial citation. There are emblematic real examples of how animals modulate nutrient cycling that would need to be explicated with much more accuracy (what was really observed and quantified by whom and where). Were there potential confounding effects that modulate the conclusions? Here of course, I think of papers by Doughty et al, 2015; Roman et al, 2010, work on dung beetles by Sitters, LeRoux et al 2020, and the corresponding reply

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by Sitters again that clearly states some pieces of the debate that can support the motive for the following study.

Also, I invite the candidate to revisit and deepen the reason underlying the lack of integration of carnivores into the picture so far. The reason mentioned in the thesis is "*because carnivores are far less abundant than herbivores*". Given the stoichiometric law, and also what is cited later on in chapter 1, based on Sterner and Elser's hypothesis and De Cuyper et al, 2020's synthesis, animals also have stoichiometric ratios that are much in balance to that of their food. They also have lower intake and gut filling. Not only are fluxes expected to be much lower, but how much of repackagers can we expect carnivores to be? Cannot carnivores be seen only as dissipators? Aren't indirect effects of carnivores likely greater than direct effects on soil OM quality? These are the types of thinking that I think have been missed in the lack of conceptualization.

At the end of the introduction, the motive for chapter 1 comes relatively naturally, i.e., getting data on the waste stoichiometry across animals, and mainly to test for the stoichiometric difference between herbivores and carnivores. But then, the natural and expected follow-up is a test on how the vertical diversity (aka the food chain length) and trophic pyramid structure are likely to affect nutrient cycling. Modelling, even simple and theoretical, is basically what I expected. The shift of focus to ecophysiology appears as a complete hiatus with the logical line of the introduction. If it was motivated by recommendations in Sitters and al, 2017, then the reader needs guidance. Chapter 3, on isotope trophic fractionation, appears as a complete outlier.

As a small, nonetheless very important note, the candidate should provide greater care in citing sources when providing a statement. For instance, the third statement in p7 is almost a copy-paste from Table 1 in Sitters et al., 2017 (although not cited here).

Chapter 1 is a meta-analysis of waste's nutrient content and stoichiometry across animal species. For me, this chapter is the most promising work of this thesis. Data were collected from the literature and complemented by data collected from zoos. Although the chapter deserves further work to reach publication standards, there is exciting potential here. Showing the lack of data, providing more of those, emphasizing the gap in non-mammals, and investigating the link with body size are all meaningful insights. Required improvements concern the transparency in the data, the introduction motivation, and the hypotheses settings. There is also incoherence between the results and the discussion. I will also dedicate a full section to Fig. 1.5. So, for now, my comments will be only for the parts on the C,N,P dataset (without the absorption efficiency dataset).

As of the introduction, there is the need to clearly state the debate around waste stoichiometry (at least for herbivores) and body size (the Leroux's versus Sitters' argument). Doing so shall strengthen the motivation of the study (because the monotonic relationship to body size is quite contested, in theory as in facts). Although there have been also previous attempts to collate such datasets (I think it is important herein to cite Reese et al, 2018 (<https://doi.org/10.1038/s41564-018-0267-7>), I would recommend clearly stating that no such dataset existed so far, while needed to solve such a puzzle, but also to reach scaling rules by which we could model ultimately the role of animals at an ecosystem scale. In the results, it is necessary, for data transparency, to show the data sources and provide a brief description of the collated dataset. ANCOVA is also the statistical analysis you are looking for. You acknowledge the low statistical power but still interpret conclusions (required control for the effect of outliers as for 1.3.c carnivores). If looking for conciseness, then I suggest non-mammals data go to supplementary, and instead provide a better description of the dataset. There are also contradictions between the results and the discussion, concerning the body mass effect on %P. The discussion on the effects of wastes

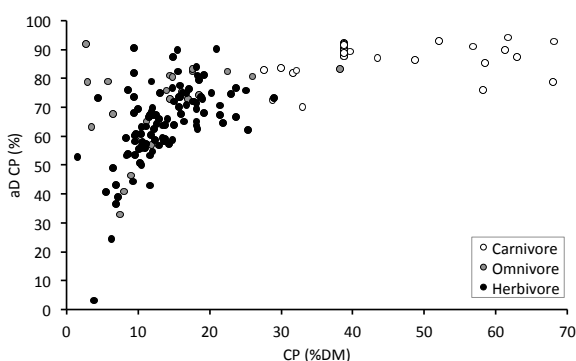
C/N/P on nutrient fate is interesting, although it would require more supporting references (so elaborate). The discussion also shows the bias of the herbivore perspective. The stoichiometry of waste is compared to that of litter (so animal waste should act as ecosystem fertilizers), but this comparison does not hold when comparing food and waste for carnivores.

Now onto fig 1.5. Based on this figure, very low dietary %N leads to negative absorption efficiency, which is interpreted as a depletion in the animals' nutrient pool (end of 1.3.2). This conclusion is also placed at the core of the final discussion of the thesis (sections 4.1 and 4.4) and fuels the candidate's idea that animals might shift from being a nutrient sink to a nutrient source at the ecosystem scale.

My first problem with this figure is that it is regarded as a result of the candidate's research process (1.3.2) while most of the dataset comes from another paper (Richard et al, 2017). The figure 1.5 is "a modified version" from Richards', complemented with new data. Based on my rough computations, new data for N represent only 5% of the individual dataset (and it seems that fig 1.5 is at the individual rather than the species level, see fig S1 from Richard's) and 7% at the species level (157 versus 170 species). Does that qualify for original results or a new analysis? I acknowledge this might not be the case for other nutrients, but for N, which is strictly equivalent to the "crude protein" compound in Richard et al, there is a real doubt here.

Besides, the fact that the apparent digestibility follows a curvilinear curve with the diet N content is not new (it is qualified as "typical" by Richard), and negative apparent digestibility is already discussed in Richard's paper. More broadly, the issue of apparent and true digestibilities, the quantification of exogenous and endogenous N sources in herbivores where N acquisition can be very critical, is a topic that has been discussed for decades (for instance [Mattson, 1980](#)). The role of coprophagy, rumen, and microbiote is also quite a developed research field (See Reese et al, 2018). As such, one more time, it shall not be regarded as a discovery from the candidate's work. This whole field of literature has not been explored despite a very strong focus of the candidate on this particular "result".

Nevertheless, this question of "negative assimilation efficiencies" and animals acting as nutrient sources on which the candidate speculates quite a lot, with very little, if not any, literature support, needs quite a critical vision. At a steady state, there is no way that an animal species acts as a source just because the nutrient pool cannot be eternally used up. The only exception is for animals whose microbiome might contain nitrogen-fixers, for instance. Then, there is the absolute need to be quite clear on the circumstances in which such data were obtained. Contrary to the treatment of the other dataset of this chapter, herein the data are individual data. Negative values of the assimilation efficiencies were likely obtained in feeding situations that would have been unsustainable over the long term. And indeed, at the species level (fig S1 Richard et al), there is no more negative assimilation efficiencies (see below, fig S1 from Richard, at the species level).



What is to the candidate's credit is to put aside such concepts within the overall framing of the role of animals' waste on terrestrial nutrient cycling. But then, this should have been set within the introduction (of the thesis and the paper), as a motive that different species, even within herbivores, may generate different effects on nutrient recycling. It might be another way to explore how some animals may play less as a sink as others, beyond the sole size effect. Whether animals' digestion results in a transient net source can be discussed, but examples must be provided. What is very harmful here is that the idea and concept remained treated superficially, occulting the abundant literature while still considered as a primary outcome of the thesis. A critical view is lacking, ending in a lot of unsupported speculation.

Finally, I will go straight to chapter 3 (I leave the chapter 2 to the other reviewer who is an expert in this field).

Chapter 3 is a feeding experiment quantifying how the isotopic trophic fractionation in N and C varies with feeding levels. This chapter is published in Peer Community Journal, so my comments will have limited impact. Experiments can be challenging, but the conduction of the experiment has been done with great care. However, I have questions about the experimental design, which is not fit to answer the questions and hypotheses as announced in the abstract and introduction. The abstract and introduction focus greatly on starvation ("starvation levels") and how it affects the isotope trophic fractionation. This is also what the person who recommended the paper in PeerC retained as the most prominent feature. Yet, the definition of starvation is not "feeding at suboptimal levels"; it is actually "full food deprivation", the absence of feeding (Cambridge Dictionary, but also Doi et al, 2017). Even the set of hypotheses mentions that the isotopic effect of starvation is linked to negative growth rates. However, animals were fed in the experimental setting, and there was no weight loss or negative growth; this is not a starvation experiment. The statement that "we show that food limitation does not always increase $\Delta^{15}\text{N}$ " is inappropriate (p56). It is an experiment measuring the effect of feeding level on isotope fractionation.

The introduction's second part mentions feeding levels' role in trophic fractionation factors. It motivates the study by stating that the effects of varying feeding levels on trophic fractionation are poorly investigated while referring to a study that dates back from 2007 (p50). This statement is quite wrong. Not only is it well studied, and so well understood that it is quite well mechanistically modeled, using notably Dynamic Isotope Budget (very similar to DEB models). I will refer the candidate to the papers by Martinez del Rio and Wolf (2005), Pecquerie et al, 2010, Emmery et al (2011). The results of the experiment are in line with what we know about the impact of feeding levels. An interesting point, yet to be mentioned or discussed, is the degree to which the isotope fractionation varies within individuals for a single treatment. The range of isotope variability is almost as high as the variability between treatments.

Variability in the trophic fractionation factor has been acknowledged years ago in ecology (Post, 2002). Therefore, ecologists have used for over a decade Bayesian estimates within mixing models and even estimation of trophic positions to account for these uncertainties and provide results assorted with confidence intervals.

To summarize, the Ph.D. thesis of S. Charberet is on a fascinating topic. The candidate had the laudable ambition to cover the subject from a broad perspective (from ecology to ecophysiology). However, this ambition's downside is that many works have not been conceived, designed, or thought in a way that

provides new knowledge (except for chapter 1), and the overall work suffers from superficial if not speculative, approaches. A major problem in the thesis is the lack of grounding within previous scientific knowledge and literature. We, researchers, all stand on the shoulders of giants; we build on the findings of many others. Knowing what has been done and learned so far is essential so our next work will advance the knowledge, not reinvent the wheel. Science can also be confirmatory and should be acknowledged as such. What can be a discovery for us has been long known by others. Second, the thesis suffers from unnecessary speculations. The basic principle of science is that how you interpret your results, especially those that appear very surprising, resists all kinds of suspicions: are there confounding factors, how good are the data, how does literature support this? Critical view is what makes strong discoveries. Unfortunately, in the thesis, this critical vision is lacking.

I am very sorry that, based on this version of the doctoral manuscript, I cannot authorize the PhD defense. I hope my comments will help to rework this document for a successful evaluation next time.

Marie-Elodie Perga

