Heavy Metal (PTE) Ecotoxicology, Data Review: Traditional vs. a Compositional Approach

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1 Heavy Metal (PTE) Ecotoxicology, Data Review: Traditional vs. a Compositional Approach

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14 Highlights

- 15 • First ecotoxicological study to compare compositional and "*traditional*" approaches.
- 16 • Both log₁₀ and clr resolve compositional trends at the point of the individual.
- 17 • The clr transformation is best able to resolve trends between PTEs.
- 18 • A compositional approach should be adopted for terrestrial ecotoxicology.

Graphical Abstract

Centred log ratio and the log10 transformation are the most congruent

Centred log ratio shows more distinct clusters than the log10 transformation

Centred log ratio open-up the data more than the log10 transformation

Abstract

Potentially Toxic Elements (PTEs) otherwise known as heavy metals are ubiquitous in soils and can have a range of negative health and environmental impacts. In terrestrial systems understanding how PTEs move in the environment is made challenging by the complex interactions within soil and the wider environment and the compositional nature of PTEs. PTEs are compositional because data of individual PTEs within a sample are ratios which may be under a sum constraint, where individual components sum up to a whole. In this study three different scenarios were considered, one using the centred log ratio transformation (clr) a compositional transformation, the more "traditional" log₁₀ transformation (log₁₀) and untransformed data acting as a comparison (unt) were applied to four different datasets. Three were the Liver, Muscle and Kidney tissue of Eurasian Badgers (*Meles meles*) and the fourth was soil and data were extracted from a regional geospatial survey. Cluster analysis demonstrated that the clr and log₁₀ transformation were able to resolve compositional trends at the point of the individual sample, while unt could not and did not meet the preconditions for the next phase of analysis. At the level of compositional trends between PTEs complex heatmaps demonstrated that clr was able to isolate PTE relationships and highlight commonalities between different datasets, whilst log₁₀ could not. In the final phase, principal component analysis (PCA) of the clr transformation showed similarities between the signals in the soft tissues and the disparities they had with soil, whilst the log₁₀ transformation was unable to achieve this. Overall, the clr transformation was shown to perform more consistently under a variety of analytical scenarios and the compositional approach will provide more realistic interpretations about PTEs in both soil and animal soft tissue than the log₁₀ or unt conditions.

Keywords: Potentially toxic element, Compositional Analysis, Compositional Trends

78 1. Introduction

79 Contamination is defined as a substance being active in a system where it does not naturally occur
80 or being present above previously recorded background levels, whilst pollution is contamination that
81 can lead to ill effects in living organisms, whether they be human or another species (Chapman,
82 2007). In the Geosciences, questions of contaminants and potential pollution are encountered often
83 and analysing their effects and movement in the environment is essential. However, a complication
84 faced by many analysts is that many data sets used in analyses are compositional in nature, where
85 compositional data are defined as multiple positive vectors being part of a whole or unit sum
86 constraint (Aitchison, 1994). These compositions are formed from separate balances and the main
87 rationale of compositional transformations is to reduce the dimensions of the data whilst retaining
88 the interconnected signal within the data (Egozcue, 2005). There are also multiple ways of
89 elucidating these compositional *fingerprints*, offering a range of tools to the potential analyst
90 (Aitchison, 2005).

91 Compositional data analysis (CoDA) approaches have developed a range log ratio
92 transformation which take account of the compositional nature of data, though the total sum may
93 not always be known but this varies between transformations (Pawlowsky-Glahn and Egozcue, 2001;
94 Egozcue et al. 2003; Tolosana-Delgado 2012; McKinley et al. 2013). Log ratio transformations reduce
95 the dimensions of a dataset and the selection of the pwlr (pairwise log ratio), alr (additive log ratio),
96 clr (centred log ratio) or ilr (isometric log ratio) transformations, is dependent on the task at hand
97 (Filzmoser and Hron, 2009). Once data are transformed, common forms of analysis are correlation
98 coefficients and principal component analysis (PCA) to assess the underlying relations between the
99 constituent parts (Kynčlová *et al.*, 2017). This is now commonly done in bioinformatics, where
100 genetic sequence data are constrained by the assay technologies ability to record counts of genetic
101 data (Quinn *et al.*, 2018). Consequently, it is these patterns of correlations that form the unique
102 *fingerprint* that can be used to interrogate complex environmental trends. This approach has had

success in geochemistry where log ratio transformations on trace elements can reveal more information about a soil horizon than the “traditional” log transformation but can also produce reliable comparisons between soil horizons (Reimann *et al.*, 2017).

1.1 Assessing Causal Trends from Compositional Signals

Assessing trends between sub-compositions remains challenging, the family of ilr transformations have been argued to be the best option when analysing compositional data as the isometric transformation removes collinearity from data which the clr transformation can produce as values scaled to sum to zero. In turn, use of an ilr transform may be particularly useful when compositions contain meaningful sub-compositions as this log-ratio can accommodate this level of complexity (Filzmoser *et al.*, 2009). This is most true of geochemical datasets comprising a high number of elements where there are multiple sub-compositions representing signals from multiple soil types and parent rocks. In ecotoxicology it is the transference of the compositional signal at source to sink which is of concern. In this case the clr transformation is helpful as this log-ratio both preserves the original variable names and reduces complexity allowing for clearer comparisons, particularly when few elements (10 or fewer) are the subject of study (Egozcue *et al.*, 2003). This is often true in the toxicological context when it is potentially toxic elements (PTEs) and not toxicologically inert elements which are the subject of study (McKinley *et al.*, 2013; Barsby *et al.*, 2012). Previously, the focus has been estimating bioavailability and bioaccessibility fractions from soil directly as well as identifying PTE sources and domains (Palmer *et al.*, 2013; McIlwaine *et al.*, 2015; Palmer *et al.*, 2015; Plouffe *et al.*, 2015). More recently assessing the causal trends between PTEs and public health data has become a high priority as CoDA can provide a degree of insight so long as the mechanisms are well understood and it is these mechanisms which impart the most knowledge in ecotoxicology (McKinley *et al.*, 2013; Jackson *et al.*, 2016; McKinley *et al.* 2020a,b).

1.2 The Application of Compositional methods in Ecotoxicology

Within the life sciences, compositional methods have been adopted in microbiology as studies produce datasets that culminate to an arbitrary total that is compositional in nature (Gloor *et al.*, 2017). For example, in shotgun metagenomics of environmental samples, the media are compositional from the point of sample collection as all members of the microbiome represent distinct parts of a whole and when DNA is dissociated from cells a reference library can be used to approximate the proportional occurrences of taxa relative to the whole solution (Calle, 2019). In ecotoxicology, there is space for compositional methods to greatly inform our knowledge of PTE accumulation but most importantly identifying associations between individual animals and PTEs is essential to identify potential mechanisms by which PTEs move through the environment. Currently, the bioaccumulation pathway (accumulation through diet) is well understood (Zietara *et al.*, 2019) and there has been research that has informed our understanding of the bioaccessibility pathway (accumulation through environmental exposure) (Miranda *et al.*, 2009). However, the standard approach in PTE ecotoxicology remains either to report totals or averages of accumulation, or the log₁₀ transformation is used to address the skew to the right, which is common in PTE data, followed by a statistical analysis. The issue is that PTEs in soil are compositional in nature and that ratio orientated signal remains after accumulation, which the log₁₀ transformation is not easily able to accommodate, as data are transformed without preserving the relative compositional signal. Hence the question remains, what are the differences between these options and what are the strengths of the compositional approach when compared to the more conventional use of the log₁₀ transformation or untransformed data ?

2. Methods

2.1 Collection of PTE Ecotoxicological Data

Badger liver, kidney and muscle (right forelimb) were dissected from road traffic accident (RTA) specimens between November 2017 and October 2018, from across Northern Ireland. Samples were stored at -20°C and then freeze dried for a period of 72 hours, homogenised with an agate pestle and mortar, 0.5g of homogenised sample was then acid digested in a digestion microwave (specification: CEM Corporation, MARS 240/50) in a solution of 10mL of HNO₃ (65% v/v), 2mL of H₂O₂ (30% v/v) digested for: 0-1600 W, 20-220°C for 30 minutes, 0 W, 20°C for 30 minutes and each digestion was run alongside a blank control and the beef reference material RM 8414 was used to validate results (Ihnat, 1994).

Analysis of samples was conducted by ICP-OES, detection limits set to 0.1 µg/mL for Al, As, Cu, Mo, Ni, Pb, Se and Zn; and 0.01ml/L for Cr and Sr respectively. All results were corrected for contamination and a dilution factor was used to take account of sample dilution; half of the detection limit was used in the case of no detectable sample.

2.2 Soil Data

The arithmetic mean of the soil data closest to the road traffic incident (within a 1.5 km radius or next closest point) that killed each individual badger was taken. The soil data was collected from the Tellus Survey, a publicly available geochemical dataset produced by the Geological Survey of Northern Ireland (Smyth, 2007; Young and Donald, 2013). All the soil samples are presumed to represent signals from the area in the badger occupied before its death.

2.3 Statistics

Cluster plots were made for each dataset, using the *CoDA* clr transformation, the log₁₀ transformation and the untransformed data (unt), using the ward.D method with Euclidean distance and Tanglegrams were produced using the package *dendextend* in R (Galili, 2015). Tanglegrams,

comparative plots between two dendrograms with the same end points, were produced from these cluster plots to demonstrate the differing degrees of congruence between the different data types within each of the four datasets (Liver, Muscle, Kidney and Soil) (Figure 1 - 4). Only the clr and log10 data were suitable for the next phase of analysis due to homogeneity in the unt dataset. Bootstrapping (random sampling with replacement) set to 1000 repetitions was used on the data (Suzuki and Shimodaira, 2006) and complex heatmaps were produced using spearman's correlation, to assess the underlying signal in the data (Reimann *et al.*, 2017). Lastly, principal component analysis was conducted, using the *princomp* function in R, to assess the differences between the clr and log10 transformations ability to separate trends of individual PTEs within the datasets (Team, 2013). These data have been published previously and for information on PTE accumulation refer to Mullineaux *et al.*, (2020).

3. Results

The Tanglegrams for the Liver (Figure 1 A), Muscle (Figure 1 B), Kidney (Figure 1 C) and Soil (Figure 1 D) datasets show high levels of congruence between the clr and log10 transformations and low levels of congruence between clr/log10 and unt. The distance metrics between clr and log10 differ by a factor of 2-3, while the unt distance metrics are much greater showing that the unt dendrograms are more poorly resolved than both the clr and log10 dendrograms. Though the clr and log10 Liver, Muscle and Kidney (Figure 1 A I, Figure 1 B I, Figure 1 C I) dendrograms show a higher degree of congruence than the equivalent soil dendrograms (Figure 1 D I). The unt dendrograms also show a "pull to the right" indicative of a poorly resolved dendrogram with a group of clades containing few leaves and a long branches indicative of outliers within that clade (Figure 1 A II/III, Figure 1 B II/III, Figure 1 C II/III, Figure 1 D II/III). Overall, unt shows signs of producing mixed signals within the data making it inappropriate for the analysis of PTEs in both organic tissue and soil. Though it should be noted that clr and log10 are both equally able to resolve the relationships of

200 accumulation between groups of individual badgers, but these diagrams do not show which
201 combinations of PTEs are contributing to these patterns.

Figure 1: Liver (A I) clr vs. log10 (A II) clr vs. unt (A III) log vs. unt. A I is most congruent, A II and A III equally so; Muscle (B I) clr vs. log10 (B II) clr vs. unt (B III) log vs. unt. B I is most congruent, B II and B III equally so; Kidney (C I) clr vs. log10 (C II) clr vs. unt (C III) log vs. unt. C I is most congruent, C II and C III equally so; Soil (D I) clr vs. log10 (D II) clr vs. unt (D III) log vs. unt. D I is most congruent, D III and then D II. Branches with solid lines represent clades which were resolved identically in both trees, whilst perforated lines represent clades resolved non-identically, thus containing individuals which did not cluster together in the corresponding dendrogram.

The homogeneity of the unt datasets prevented them from being included in the next phase of analysis and correlation heatmaps were created for both clr and log10 following the method described by Reimann (2017). All elements are included in the clr heatmaps due to the compositional approach being able to estimate values for missing data given that all values are treated as inter-related ratios. Whilst As is excluded from Figure 2 B, D and F, Pb is also excluded from Figure 2 D as log 10 cannot account for missing data, whilst all elements remain in Figure 2 A, C, E, G and H. However, though clr can account for missing data, that alone is not cause for its inclusion, the adjoining trees in Figure 2 C and D as well as E and F show that for Muscle and Kidney both clr dendrograms have shown a pull to the right and that is not seen in the equivalent log10 trees showing the signal remains stronger due to the exclusion of missing data.

Throughout all PTE clr dendrograms and heatmaps distinct patterns can be seen (Figure 2 A, C, E, G). In the three tissue plots Al, Cu, Cr and Sr can be seen to cluster together consistently and the same cluster is seen in the clr soil tree with the inclusion of Ni Se and Zn cluster consistently in all clr dendrograms with the inclusion of Mo in the clr Liver plot and the inclusion of As and Pb in clr Muscle plot. As and Pb also cluster together consistently in all three tissue dendrograms with the inclusion of Mo in the clr Soil plot. Mo and Ni are also closely associated and cluster together in both the clr Muscle, and Kidney dendrograms and are closely associated in paired groups in the clr Liver dendrogram. However, both Mo and Ni are at opposite ends of the clr Soil dendrogram and this is

the only observable deviation between the clr tissue dendrograms and the clr Soil dendrogram (Figure 2 A, C, E and G). These shared patterns between the clr plots are not seen in the log10 plots (Figure 2 B, D, F and H), Al, Cu, Cr and Sr cluster closely in these dendrograms, but it is not as clear with the inclusion of Ni in the log10 Liver plot, Zn in the clr Muscle plot and dissociation in the log10 Kidney and Soil plots. Se and Zn only cluster in the log10 Liver and Kidney plots and are dissociated in the log10 Muscle and Soil plots, both also cluster in the soil plots. As is excluded from all log10 tissue dendrograms and Pb from the log10 Muscle dendrogram, so the As and Pb cluster did not have the opportunity to arise under the log10 approach. However, As and Pb have clustered with Mo and Se in the log10 soil plot, and Mo is associated with As and Pb in the clr Soil dendrogram. Mo and Ni only cluster together in the log10 Muscle dendrogram and are disassociated in all other log10 dendrograms (Figure 2 B, D, F and H).

Figure 2: (A) clr Liver, (B) log10 Liver, (C) clr Muscle, (D) log10 Muscle, (E) clr Kidney, (F) log10 Kidney, (G) clr Soil, (H) log10 Soil. The green dotted lines in each branch correspond to 0 and the solid green line shows the degree of shift from the 0 value, as shown in the density plot.

Principal Component Analyses of clr and log10 were performed with the same exclusions for log10 as the prior analysis and both scree and biplots are presented (Figure 3). The clr PCAs have captured more variance than the log10 PCAs, as higher degrees of variance are seen under clr in PC1 and PC2 than log10 (Figure 3 A, C, E and G; Figure 4 A, C, E and G). The clr Liver, Muscle and Kidney biplots are highly similar in the directionality of the elements, though this relationship is not shared with soil (Figure 3 B, D, F and H). However, for log10, Muscle and Kidney show a similar pattern of directionality, but Cu is more prominent in the Liver under log10, like clr, no clear relationship can be observed with Soil (Figure 4 B, D, F and H). Soil under clr, shows multi-directionality in the eigenvectors, whilst under log10, the eigenvectors are more correlated (Figure 3 H; Figure 4 H). More variance is accounted for by the clr transformation in all scenarios, but no clear similarities can be seen with soil under both transformations. Overall, the angles between the eigenvectors are more obtuse under clr and more acute under log10. Thus, demonstrating that the compositional relationships between elements have been more clearly resolved using clr rather than log10.

Like the previous analysis there are consistent patterns between the PTEs. In the clr PCA biplots the eigenvectors for Al, Cr, Cu and Sr have the longest eigenvectors, indicating they are consistently contributing the highest degrees of variance to PCs 1 and 2 in these analyses with the addition of Ni in the clr Kidney and Soil plots. Se and Zn remain closely affiliated across all clr biplots indicating a strong correlation but are associated with Mo in the clr Liver plot, associated with As, Ni and Pb in Muscle plot (likely due to reduced signal in this tissue type) and remain distinct in the clr Soil biplot. As and Pb are also closely associated across all clr biplots, showing a correlation and have short eigenvectors indicating they contribute a low degree of variance to PCs 1 and 2, though they are associated with Ni in the clr Liver biplot, Ni, Se and Zn in the clr Muscle biplot and Mo in the clr

Soil biplot (Figure 3 B, D, F and H). Decoding the magnitude of variance contribution and correlations between eigenvectors is made possible by these clr biplots, demonstrating the compositional relationships between the eigenvectors (Tolosana-Delgado, and McKinley, 2016). In the log10 biplots Al, Cr, Cu and Sr remain dominant and in the log10 Muscle and Kidney biplots, all other elements show mono directionality indicating that for these datasets log10 was not able to separate the compositional signals of these elements. In the log10 Liver plot the eigenvectors for Mo, Se and Zn are closely affiliated and Ni and Pb are also closely affiliated. In the log10 soil biplot, the eigenvectors for Al, Cr, Cu, Ni and Sr are closely affiliated, As, Mo and Pb are closely affiliated and Zn is between these two groupings (Figure 4 B, D, F and H) (Supplementary Material, Table S1-S8).

339 Figure 3: (A) clr Liver scree plot, (B) clr Liver biplot, (C) clr Muscle scree plot, (D) clr Muscle biplot, (E)
340 clr Kidney scree plot, (F) clr Kidney biplot, (G) clr Soil scree plot, (H) clr Soil biplot.

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Figure 4: (A) log₁₀ Liver scree plot, (B) log₁₀ Liver biplot, (C) log₁₀ Muscle scree plot, (D) log₁₀ Muscle biplot, (E) log₁₀ Kidney scree plot, (F) log₁₀ Kidney biplot, (G) log₁₀ Soil scree plot, (H) log₁₀ Soil biplot.

4. Discussion

In this comparison, it can be qualitatively noted that the clr transformation has “opened up” the data more effectively than log₁₀, even though both transformations produce highly congruent dendrograms (Figure 1 A, 2 A, 3 A) clr retains a higher distance metric showing that more variance has been preserved in the data and the data are not “over-corrected” as the compositional balances in the data have been considered (Aitchison, 1982). Another advantage remains that clr data can account for a variable with many values represented by half the detection limit. However, the norm in the geosciences is that variables with far too few observations should be removed from the analysis but in the context of ecotoxicology where a source (soil) and sink (badger) need to be considered in parallel, patterns cannot be assessed comparatively if elements are removed from tissue data, for it is similarities and deficiencies in the compositions that need to be considered when assessing trends of accumulation. These trends can be compared in the biplots of the PCA analysis which is a standard approach in compositional analysis (Figure 3) (Aitchison and Greenacre, 2002; Aitchison, 1983). Though, it should be noted that the algebraic descriptions and resulting geometries of each data type are not directly comparable and that the results are a demonstration of the differences between these approaches,

When compared, the clr complex heatmap plots show a more consistent pattern than their log₁₀ equivalents (Figure 2). The clr transformation allows for the compositional patterns to be identified, as shown by the angles in the eigenvectors (Figure 3 B, D, F and H; Figure 4 B, D, F and H), and these distinct clusters can identify source to sink trends (Egozcue and Pawłowsky-Glahn, 2019). These groupings are Al, Sr, Cr and Cu; Mo, Se and Zn; and As, Ni and Pb. The Al, Sr, Cr and Cu

signal can be attributed to the mafic zone in the North East of Northern Ireland with potentially some influence from the granitic Mourne Mountains in the South. The Mo, Se and Zn signal can be attributed to the Silurian greywacke and Shale of the South East and Ards Peninsula which is known to be a mineralising zone. The As, Ni and Pb signal could be attributed to the under sampling seen in the tissue data creating homogeneity between these variables, though there is likely an anthropogenic signal in this grouping associated with urbanisation and industrialisation in the East and South East of Northern Ireland (Smyth, 2007). These clusters are identifiable in both the badger tissue and soil data and demonstrate how the compositional approach can show this source too sink relationship.

4.1 Compositional Analysis in the Life Sciences

Compositional analysis is not new to the life sciences, recently the compositional approach has been adopted in microbiology as species present in a microbiome collate to a total imposed on the data by the instrument used (Gloor *et al.*, 2017; Rivera-Pinto *et al.*, 2018) and alternative compositional frameworks have been designed (Brill *et al.*, 2019). In the experimental context of microbiology, the compositional approach has been applied to understanding cellular activity, given a proportionate baseline one can measure what is changing in relation to a cause of change (Quinn *et al.*, 2019). This philosophy has been applied successfully to understand changes in microbial communities in relation to factors like temperature in alpine habitats, seasonality in marine systems and the evolution of dental calculus microbiomes and how they vary temporally (Frindte *et al.*, 2019; Aluladell *et al.*, 2018; Brealey *et al.*, 2019). In turn, there is a basis for the compositional approach in the life sciences and as demonstrated here it offers important insights in an ecotoxicological context (Mullineaux *et al.*, 2020).

4.2 Compositional Analysis in the Geosciences and Medical Geology

In the geosciences, compositional methods are well established and have opened the possibility for clearer assertions to be made about soils and environmental pollution, allowing for causative trends

to be identified (Aitchison, 1994; Buccianti *et al.*, 2006). Such causative trends are identified in the field of medical geology, where large amounts of public health data, when made accessible, can be used to evaluate the risks of pollutants and contaminants (McKinley *et al.*, 2013; McKinley *et al.* 2020a, b). However, in ecotoxicology, it can be difficult to identify the mechanisms through which pollutants and contaminants move through the environment, and it is here where the compositional approach offers more power. Especially, as demonstrated above, where ratios at source can be matched with those at sink placed in a wider geospatial context. In medical geology, by measuring concentrations of individual PTEs and how they co-vary (McIlwaine *et al.*, 2015), combining this information with bioaccessibility quotients (Barsby *et al.*, 2012; Palmer *et al.*, 2013; Palmer *et al.*, 2015) and utilising public health data or another metric, compositional methods can identify specific pollutants and contaminants which are potentially causal for various health conditions (Jackson *et al.*, 2016). Therefore, in PTE ecotoxicology, if sources and sinks are to be identified effectively, patterns of accumulation, trends and mechanisms identified, then compositional methods should be considered an effective tool in an analyst's arsenal. Consequently, as demonstrated here understanding processes of PTE accumulation requires accounting for the compositional signals within PTE data.

5. Conclusion

The log₁₀ transformation may be acceptable for understanding PTE accumulation patterns at the point of the individual as demonstrated by the high degrees of congruence between log₁₀ and clr for individual badgers (Figures 1 A-D). However, it is not acceptable when looking at patterns at the population level when looking to elucidate trends between PTEs (Figure 2). This is further seen when looking at the compositional patterns of PTEs between individual tissues and soils (Figure 3 and 4) because the clr transformation highlights the similarities between the tissue types and the disparity they have with soil most clearly. Overall, the clr transformation is clearly better at isolating the

compositional trends, hence allowing for more precise inferences about the accumulative mechanisms of PTEs from soils to the biosphere.

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586 Table S1: Liver clr PCA output.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Standard Deviation	1.5004639	1.0261168	0.8444990	0.60227493	0.41041880	0.25735762	0.22910929	0.131621914	0.114673283	5.875111e-09
Proportion of Variance	0.4792374	0.2241265	0.1518091	0.07721278	0.03585536	0.01409853	0.01117339	0.003687704	0.002799137	7.347369e-18
Cumulative Proportion	0.4792374	0.7033640	0.8551731	0.93238588	0.96824124	0.98233977	0.99351316	0.997200863	1.000000000	1.000000e+00

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588

589 Table S2: Muscle clr PCA output.

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	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Standard Deviation	1.697437	1.3614065	0.68496000	0.3587262	0.23659803	0.210074483	0.16760999	0.08793572	1.199301e-08	5.126317e-09
Proportion of Variance	0.526888	0.3389273	0.08579488	0.0235319	0.01023654	0.008070075	0.00513725	0.00141404	2.630193e-17	4.805535e-18
Cumulative Proportion	0.526888	0.8658153	0.95161019	0.9751421	0.98537864	0.993448710	0.99858596	1.00000000	1.000000e+00	1.000000e+00

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598 Table S3: Kidney clr PCA output.

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	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Standard Deviation	1.4833993	1.3559653	0.7905447	0.66846109	0.46496486	.27508141	0.24430141	0.164692634	0.12536473	1.071725e-08
Proportion of Variance	0.3997008	0.3339766	0.1135198	0.08116544	0.03926984	0.01374489	0.01084104	0.004926826	0.00285476	2.086341e-17
Cumulative Proportion	0.3997008	0.7336774	0.8471972	0.92836265	0.96763249	0.98137738	0.99221841	0.997145240	1.00000000	1.000000e+00

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601

602 Table S4: Soil clr PCA output.

603

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Standard Deviation	0.9521091	0.4400194	0.4043468	0.28925893	0.28925893	0.26672038	0.18464787	0.14308542	0.1052076	4.998999e-09
Proportion of Variance	0.5643829	0.1205436	0.1017908	0.07603491	0.05209236	0.04429073	0.02122702	0.01274651	0.0068912	1.555846e-17
Cumulative Proportion	0.5643829	0.6849265	0.7867173	0.86275219	0.91484454	0.95913527	0.98036229	0.99310880	1.0000000	1.000000e+00

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610 Table S5: Liver log10 PCA output.

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	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Standard Deviation	0.6883491	0.4847523	0.4431278	0.28439448	0.20530257	0.12568370	0.10428894	0.077093756	0.056985312	0
Proportion of Variance	0.4452969	0.2208370	0.1845398	0.07601065	0.03961146	0.01484534	0.01022136	0.005585609	0.003051814	0
Cumulative Proportion	0.4452969	0.6661340	0.8506738	0.92668442	0.96629588	0.98114122	0.99136258	0.996948186	1.000000000	1

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613

614 Table S6: Muscle log10 PCA output.

615

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Standard Deviation	0.8330142	0.6087193	0.29815709	0.16671896	0.13650615	0.101160670	0.072834422	0.046432140	0	0
Proportion of Variance	0.5699614	0.3043511	0.07301818	0.02283025	0.01530542	0.008405511	0.004357266	0.001770835	0	0
Cumulative Proportion	0.5699614	0.8743125	0.94733072	0.97016097	0.98546639	0.993871899	0.998229165	1.000000000	1	1

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622 Table S7: Kidney log10 PCA output.

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	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Standard Deviation	0.7258514	0.6128254	0.3543896	0.29853939	0.25765593	0.15895068	0.11937154	0.095021267	0.054517218	0
Proportion of Variance	0.4265952	0.3040843	0.1016910	0.07216454	0.05375277	0.02045716	0.01153778	0.007310754	0.002406511	0
Cumulative Proportion	0.4265952	0.7306795	0.8323705	0.90453503	0.95828780	0.97874496	0.99028273	0.997593489	1.000000000	1

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625

626 Table S8: Soil log10 PCA output.

627

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Standard Deviation	0.4840696	0.3915002	0.18868389	0.15365391	0.13146106	0.11715693	0.09900876	0.08018314	0.060174697	0.045424239
Proportion of Variance	0.4688989	0.3067098	0.07124153	0.04724446	0.03458262	0.02746627	0.01961602	0.01286559	0.007245885	0.004128944
Cumulative Proportion	0.4688989	0.7756087	0.84685022	0.89409468	0.92867730	0.95614356	0.97575958	0.98862517	0.995871056	1.000000000

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