

Molecular diversity as a biosignature

Corresponding Author: Dr Gideon Yoffe

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 1:

Reviewer comments:

Reviewer #1

(Comments for the Author)

General comment: with a background in microbiology, I am not familiar with some of the analysis/statistics that the authors did. Nevertheless, I appreciated that authors put effort into including a large set of samples, as well as consider degradation's impact. I would have appreciate having a clearer definition of the samples and term ecodiversity from the beginning of the paper. Not clear also for me how they used the microorganisms in their study (just for fatty acids analysis or amino acids analysis as well?).

1. Summary of the key results: the authors present key results about using differential amino acids composition as a biosignature. They convinced well the reader that this technique is robust as it worked on a variety of samples and also under degradation's context. However, I would have appreciate a better link between their results obtained with the amino acids and fatty acids and take into account more clearly impact of the microorganisms.

2. Regarding originality and significance: the authors should highlight more clearly the previous papers published on the same topic, like:

- McDonald, G. D., & Storrie-Lombardi, M. C. (2006). Amino acid distribution in meteorites: diagenesis, extraction methods, and standard metrics in the search for extraterrestrial biosignatures. *Astrobiology*, 6(1), 17-33.
- Creamer, J. S., Mora, M. F., & Willis, P. A. (2017). Enhanced resolution of chiral amino acids with capillary electrophoresis for biosignature detection in extraterrestrial samples. *Analytical chemistry*, 89(2), 1329-1337

However, compared to previous publications, here the authors consider many different samples and clearly provide new results as they also account for the influence of degradation process.

3. Data and methodology: I would highlight more the diversity of samples used in the study and explain more in detail types of samples considered (e.g., Table 1 should be more at the beginning).

4. Appropriate use of statistics and treatment of uncertainties: not familiar with.

5. Conclusions: the authors highlight the robustness of their method even when considering degradation, but they should add that it concerns only one degradation process (radiolytic degradation).

6. Suggested improvements:

- Include other factors of degradation might improve robustness of the technique.
- Emphasize more the impact of microbial metabolisms: not clear for me if the samples considered as "biotic" in this study include both microbial cells themselves and samples from the surrounding environment of microorganisms (as microbial activity might change the composition of aa around). Check: Schwendner, P., Riedo, A., Melton, D. J., Horvath, P., Lindner, R., Ehrenfreund, P., ... & Cockell, C. S. (2022). Microbial metabolism of amino acids—biologically induced removal of glycine and the resulting fingerprint as a potential biosignature. *Frontiers in Astronomy and Space Sciences*, 9, 781542
- Related to previous comment: if not already done, use cells of *E. coli*, *A. tumefaciens*, *C. butyricum* and *S. marscens* for the analysis of aa composition, as they did for fatty acids.
- Include some microorganisms relevant in astrobiology (methanogens, *Deinococcus radiodurans*)

7. References: include previous works done (see point 2 above). As the authors mention (homo)chirality as a possible biosignature, they should also cite some papers working on this (e.g., Patty, C. L., Ten Kate, I. L., Sparks, W. B., & Snik, F. (2018). Remote sensing of homochirality: a proxy for the detection of extraterrestrial life. In *Chiral Analysis* (pp. 29-69).

Elsevier.)

8. Clarity and context:

- Give more context about the degradation's influence (is it only radiolytic degradation that matters? Any other?).
- For the people that are not familiar with this domain, the introduction should better explain the term ecodiversity and how they consider richness and evenness. (super well described in the method section).

(Remarks on code availability)

Reviewer #2

(Comments for the Author)

1. Summary of the key results

This study proposes the statistical structure of molecules in a sample as a class of biomarkers for differentiating between abiotic and biotic organics in extraterrestrial samples. The authors applied ecodiversity statistics to organics, instead of species, measuring richness (number of individual molecules) and diversity (uniformity of their relative abundances), then using this information to create an evenness curve (distributional structure for comparing samples with different richness). Data was collected from published studies and includes the number and abundance of individual amino acids or fatty acids from each sample (34 total samples for amino acids, eight total samples for fatty acids) of biotic, abiotic, or mixed origin. The samples were compared against each other through computation of pairwise distances between evenness curve distributions. The authors report that biotic and abiotic amino acid profiles are differentiable from each other with a k-Nearest-Neighbors classification accuracy of 86- 93%, and that within the biotic and mixed origin samples, dissimilarities between evenness curves show samples grouped into highly diverse, degraded/contaminated, or severely degraded, where the most degraded plot towards abiotic samples. The authors also simulated expected degradation rates for three selected compounds under Europa-like conditions, based on radiolytic constants, to assess whether biogenicity or abiogenicity could be determined after degradation, and found that degraded samples were still differentiable. Biotic amino acids displayed greater internal evenness with more uniform abundances, compared to abiotic samples that are sparse and favor smaller compounds. The same test was applied to fatty acids and the authors report that their statistical approach differentiates biotic and abiotic origin, however, the sample set only includes eight total samples with two synthetic abiotic samples and no natural abiotic samples.

2. Originality and significance

Statistical analysis of richness and uniformity is a clever approach for differentiating biotic versus abiotic organics, and the amino acid results show promise. However, as discussed above, a larger sample set is needed to draw firm conclusions. The fatty acid results are not significant due to the small dataset and lack of natural samples. The radiation results also require more data; including a greater number of molecules beyond the three modeled would be helpful, and any laboratory experiment data from published studies would also be a good addition. The authors also fail to mention or cite the work that has been done with molecular approaches for differentiating biotic from abiotic organics, which is essential to contextualize their results and provide justification for why their approach is ideal (references listed in the "References" segment below).

3. Data & methodology

The authors' approach is valid and promising for amino acids, but data and methodology for the fatty acid and radiation parts are lacking. In general, more samples are needed, and since calculation of molecular profiles leverages data that is already published and doesn't require physical analysis of samples, expanding the dataset is a reasonable ask. For the radiation part, the ecosystem diversity metrics do not seem applicable because only three select molecules were modeled, whereas the amino and fatty acid calculations use full suites of molecules, which is key for ascertaining diversity versus richness.

4. Appropriate use of statistics and treatment of uncertainties

The ecodiversity approach to analysis of molecular data for amino and fatty acids shows promise and is unique in its ability to capture diversity and richness in one metric. The statistical tests (e.g., Mann-Whitney U, KS, AUC, Kolmogorov-Smirnov, functional ANOVA) discussed in the supplemental are applied appropriately.

5. Conclusions: robustness, validity, reliability

The authors' results from amino acid analyses provide a compelling argument for evenness as a potential indicator of biogenicity or abiogenicity, and I agree that this could be a promising detector-agnostic metric for assessing diversity and richness. However, I would have liked to see a lot more samples, considering the input data for each individual sample was a discrete list of molecular abundances collected from published literature, so there is plenty more to pull from and doesn't require additional experimental analyses. I would especially have liked to see more data in the abiotic category – even within the selected studies there are several other meteorite samples with amino acid data reported, and it's unclear why the authors chose to exclude those ones. On the biotic side, I find it intriguing that the severely altered samples plot towards abiotic, which reaffirms expectations that diagnosticity is lost with processing. I enjoyed the sample-by-sample discussion of outliers in the "Resolving Biotic Sample Histories" section, and think that a larger sample suite that includes more modern or

fresh material could help deconvolve the trends and outliers further, especially if those samples are dominated by microbial life.

While the amino acid analyses are promising, I do not find the fatty acid analysis to be publication-worthy at this stage. Eight total samples with two synthetic abiotic samples is too small of a dataset to draw conclusions from, especially considering that both abiotic samples are synthetic. Of course, indigenous long-chain fatty acids are not present in meteorites, but shorter-chained species C1- C12 are, and the authors could pull data from these studies. This sort of test is needed to validate the model's efficacy, since this type of statistical analysis is blind to the identity of individual species within one type of compound class, and carboxylic/fatty acids with differing chain lengths are present in both biotic and abiotic natural samples. For astrobiological relevance, a reference that the authors may want to consult is Deamer et al. (1986), who showed that carboxylic acids C8+ extracted from meteorites form protomembrane-like structures. For the biotic fatty acids, the authors should include data from geologic samples instead of just individual organisms, because mixed input and varying states of degradation yield different profiles and are much more astrobiologically relevant.

I agree that it's important to consider how the effects of radiation could skew a sample's organic distribution, but more data is needed before the authors can conclude that biotic and abiotic will be differentiable, beyond modeling of three selected amino acids. The ecodiversity statistical approach does not seem applicable if only three compounds are sub-selected, since an overall view of richness versus diversity is required to make that assessment. I was also confused by the "Model Description" section in the supplemental, because as written, the authors applied radiolytic decay models to every single biotic sample in the dataset, and I'm unclear on how this translates to Figure 8 in the supplemental and Figure 5 in the main text.

6. Suggested improvements

As mentioned in previous comments, the work would greatly benefit from more samples. On the abiotic amino acid side, it would have been useful for the authors to note the meteorite's group (CI, CM, CO, CV, CK, CR, CH, CB), because amino acid content can vary across these categories. The authors should also consider that Bennu and Ryugu are both CI-type bodies, which are both unique and rare amongst meteorites (Orgueil being an exception), but they are also returned asteroid samples, so the difference in organic content could be due to the friable nature of this material and/or alteration that occurs during meteoritic atmospheric entry. For the biotic amino acid side, modern samples and samples that are dominated by microbial input are crucial additions, especially to support the application to Europa, where simple extant life is a consideration. The radiation part should be re-thought; as mentioned in my comments under the "Data and Methodology" segment, selecting three amino acids and applying degradation models does not fit with the ecosystem diversity statistical approach because it inherently eliminates the ability to accurately measure both diversity and richness.

7. References

In lines 36- 38, Napier, W. A (2004) is incorrectly cited – this paper discusses panspermia, or the concept that organisms that originated on one body could be transferred to another body, where they could be reanimated and form the basis for an independent ecosystem. The language, "widespread presence of organic molecules in extraterrestrial bodies... suggest[s] life's precursors arrived via meteorites and comets" is not panspermia. Instead, cite one of the following:

Pizzarello, S. (2006). The chemistry of life's origin: a carbonaceous meteorite perspective. *Accounts of Chemical Research*, 39(4), 231-237.

Osinski, G. R., Cockell, C. S., Pontefract, A., & Sapers, H. M. (2020). The role of meteorite impacts in the origin of life. *Astrobiology*, 20(9), 1121-1149.

Sandford, S. A., Nuevo, M., Bera, P. P., & Lee, T. J. (2020). Prebiotic astrochemistry and the formation of molecules of astrobiological interest in interstellar clouds and protostellar disks. *Chemical reviews*, 120(11), 4616-4659.

Deamer, D., Dworkin, J. P., Sandford, S. A., Bernstein, M. P., & Allamandola, L. J. (2002). The first cell membranes. *Astrobiology*, 2(4), 371-381.

The authors do not reference any of the published work on molecular approaches for differentiating between biotic and abiotic organics. A subset of this body of work is listed below:

Teece, B. L., Beaty, D. W., Graham, H. V., McDonnell, G., Sherwood Lollar, B., Siljeström, S., ... & Mackelprang, R. (2025). The abiotic background as a central component of a sample safety assessment protocol for sample return.

Buckner, D. K., Anderson, M. J., Wisnosky, S., Alvarado, W., Nuevo, M., Williams, A. J., ... & Wilhelm, M. B. (2024). Quantifying Global Origin-Diagnostic Features and Patterns in Biotic and Abiotic Acyclic Lipids for Life Detection. *Astrobiology*, 24(1), 1-35.

Dorn, E. D., Nealson, K. H., & Adami, C. (2011). Monomer abundance distribution patterns as a universal biosignature: examples from terrestrial and digital life. *Journal of molecular evolution*, 72(3), 283-295.

Saeedi, D., Buckner, D., Walton, T. A., Aponte, J. C., & Aghazadeh, A. (2025). Discriminating abiotic and biotic organics in meteorite and terrestrial samples using machine learning on mass spectrometry data. *PNAS nexus*, 4(11), pgaf334.

Johnson, S. S., Anslyn, E. V., Graham, H. V., Mahaffy, P. R., & Ellington, A. D. (2018). Fingerprinting non-terran biosignatures. *Astrobiology*, 18(7), 915-922.

Sherwood Lollar, B., Westgate, T. D., Ward, J. A., Slater, G. F., & Lacrampe-Couloume, G. (2002). Abiogenic formation of alkanes in the Earth's crust as a minor source for global hydrocarbon reservoirs. *Nature*, 416(6880), 522-524.

Cleaves, H. J., Hystad, G., Prabhu, A., Wong, M. L., Cody, G. D., Economou, S., & Hazen, R. M. (2023). A robust, agnostic molecular biosignature based on machine learning. *Proceedings of the National Academy of Sciences*, 120(41), e2307149120.

Mißbach, H., Schmidt, B. C., Duda, J. P., Lünsdorf, N. K., Goetz, W., & Thiel, V. (2018). Assessing the diversity of lipids formed via Fischer-Tropsch-type reactions. *Organic Geochemistry*, 119, 110-121.

Glavin, D. P., Burton, A. S., Elsila, J. E., Aponte, J. C., & Dworkin, J. P. (2019). The search for chiral asymmetry as a potential biosignature in our solar system. *Chemical reviews*, 120(11), 4660-4689.

8. Clarity and context

(Remarks on code availability)

I am able to access the site and view the data in CSV format, but I do not have the expertise to run the code myself.

Version 2:

Reviewer comments:

Reviewer #1

(Comments for the Author)

I thank the authors for taking into account all my suggestions and include them in their paper. For me, now the article is much easier to read/understand and can definitely be published in the journal *Nature astronomy*.

Here are some minor remarks:

3. Data and methodology:

Much clearer like this. Thanks for adding the small paragraph at the beginning of amino acid subsection. I understand that it might make the table 1 even longer, but maybe it would be good to include the per-sample label as well in this table?

7. References:

Not sure about where you included the reference about the work done by Patty et al. It is with the line talking about homochirality itself, but not the method we can use to detect it (circular polarization).

(Remarks on code availability)

Reviewer #2

(Comments for the Author)

1. Summary of the key results

This study proposes the statistical structure of molecules in a sample as a class of biomarkers for differentiating between abiotic and biotic organics in extraterrestrial samples. The authors applied ecodiversity statistics to organics, instead of species, measuring richness (number of individual molecules) and diversity (uniformity of their relative abundances), then using this information to create an evenness curve (distributional structure for comparing samples with different richness). Data was collected from published studies and includes the number and abundance of individual amino acids or straight chain fatty acids from each sample of biotic, abiotic, or mixed origin. The samples were compared against each other through computation of pairwise distances between evenness curve distributions. The authors report that biotic and abiotic amino acid profiles are differentiable from each other with a k-Nearest-Neighbors classification accuracy of 86- 93%, and that within the biotic and mixed origin samples, dissimilarities between evenness curves show samples grouped into highly diverse, degraded/contaminated, or severely degraded, where the most degraded plot towards abiotic samples. The authors also simulated expected degradation rates for amino acids under Europa-like conditions, based on radiolytic constants, to assess whether biogenicity or abiogenicity could be determined after degradation, and found that degraded samples were still differentiable. Biotic amino acids displayed greater internal evenness with more uniform abundances, compared to abiotic samples that are sparse and favor smaller compounds. A demonstration of concept was applied to a smaller set of straight chain fatty acids and the trend was reversed: biotic and abiotic compounds are differentiable, while biotic fatty acids displayed less diversity which reflects life's preference for a more limited set of isomers.

2. Originality and significance: if not novel, please include reference

I appreciate the authors' expansion of their datasets, and agree that that the results are original and significant. I also

appreciate the added references to previous work differentiating biotic from abiotic organics. However, I would like to see a little more text discussing the authors' results within the context of that body of literature. I recognize space is limited, so even a short paragraph in the discussion section would do. My reasoning is that I believe this will strengthen the applicability of their approach and properly contextualize the results – previous papers have demonstrated that biotic and abiotic organics differ, and the unique methodology in this work reiterates those previous findings while providing a complementary approach that does not rely on identification of specific molecular structures.

3. Data & methodology: validity of approach, quality of data, quality of presentation

The additional samples and analyses that the authors have included have provided added value and enhanced the robustness of the results. Regarding the fatty acids, I wonder why branch chained compounds were excluded. I understand the argument that straight chained compounds are preferred by biology for membrane formation, but cyclic and unsaturated fatty acids were included within the biotic set, and many microbes synthesize methyl branched fatty acids for similar structural enhancements. A driving argument for the statistical approach is that identification of individual molecular structures is not required, so including branched compounds is important, especially because the amino acid analysis included all isomers identified within a sample set, regardless of similarities in molecular weight. The authors mention that differing abiotic fatty acid reaction pathways produce straight versus branched compounds, but natural samples can contain input from multiple sources, so adding this tweak would make the results even more applicable to analysis of samples from other planetary bodies.

4. Appropriate use of statistics and treatment of uncertainties

The ecodiversity approach to analysis of molecular data for amino and fatty acids shows promise and is unique in its ability to capture diversity and richness in one metric. The statistical tests (e.g., Mann-Whitney U, KS, AUC, Kolmogorov-Smirnov, functional ANOVA) discussed in the supplemental are applied appropriately.

5. Conclusions: robustness, validity, reliability

I greatly appreciate the additional samples that the authors included and believe this makes their conclusions more robust.

6. Suggested improvements: experiments, data for possible revision

I only have a few minor suggestions, as I feel the authors have done a great job of expanding their analyses from my previous comments.

- As mentioned in the "Originality and significance" section, I would appreciate contextualization of their results with a short discussion of past work with traditional molecular methods. In my opinion, this addition would provide an enhanced argument for organics-based life detection efforts because it demonstrates that this type of biosignature contains a great deal of information. For astrobiology mission applications, this can help demonstrate that organic analyses are crucial, because biotic and abiotic compounds can be differentiated through either molecular identification, statistical approaches, or both.

- As mentioned in the "Data & methodology" section, I would like to see branched fatty acids included. Hopefully this will not be too heavy of a lift!

- I recommend a different color scheme for Figure 5 – it's hard to see the difference between "biotic" and "degrading biotic."

- The caption for Figure 3 could use more information. Alternatively, the authors could combine Figure 2 and 3 to avoid repeating text.

- This is very minor, but the term "panspermia" in the introduction section needs to be removed. Panspermia is the idea that viable organisms were transported from one planetary body to another, not that molecules delivered exogenously contributed to an independent origin of life event.

7. References: appropriate credit to previous work?

I appreciate the added references and only recommend a brief discussion, as mentioned above.

8. Clarity and context: lucidity of abstract/summary, appropriateness of abstract, introduction and conclusions

The work is clear and contextualized. Overall, I appreciate the authors' thoughtful additions to this version of the manuscript and feel the work is greatly improved and will be an important contribution to the field.

(Remarks on code availability)

I am able to access the site and view the data in CSV format, but I do not have the expertise to run the code myself.

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NATASTRON-25070921A Rev-1 Reply to Reviewers

We thank the reviewers for their careful reading of the manuscript and for their constructive and insightful comments. Their feedback substantially improved the clarity, scope, and rigor of this work and strengthened both the presentation and the interpretation of the results. Below, we address each reviewer's comments in detail.

All revised/added text in the manuscript is highlighted in bold.

Reviewer #1 (Comments for the Author):

General comment: with a background in microbiology, I am not familiar with some of the analysis/statistics that the authors did. Nevertheless, I appreciated that authors put effort into including a large set of samples, as well as consider degradation's impact. I would have appreciate having a clearer definition of the samples and term ecodiversity from the beginning of the paper. Not clear also for me how they used the microorganisms in their study (just for fatty acids analysis or amino acids analysis as well?).

1. Summary of the key results: the authors present key results about using differential amino acids composition as a biosignature. They convinced well the reader that this technique is robust as it worked on a variety of samples and also under degradation's context. However, I would have appreciate a better link between their results obtained with the amino acids and fatty acids and take into account more clearly impact of the microorganisms.

REPLY: Thank you for this suggestion. We now make the amino-acid and fatty-acid results directly comparable by framing them as complementary microbial constraints: amino acids reflect broad, relatively even metabolic inventories, whereas fatty acids reflect biosynthetic and membrane constraints that impose structured chain-length patterns. We also clarify that microorganisms contribute directly to both datasets (whole-cell amino-acid profiles and, where available, fatty-acid profiles). Because both classes are separated by evenness, the signal is not specific to one compound class. Given the more limited fatty-acid coverage, we present that analysis as a proof-of-concept complement, with details in the Extended Data. This is discussed in the new "Fatty acids" subsection in the "Mapping sample dissimilarities across origins" section.

2. Regarding originality and significance: the authors should highlight more clearly the previous papers published on the same topic, like:

- McDonald, G. D., & Storrie-Lombardi, M. C. (2006). Amino acid distribution in meteorites: diagenesis, extraction methods, and standard metrics in the search for extraterrestrial biosignatures. *Astrobiology*, 6(1), 17-33.
- Creamer, J. S., Mora, M. F., & Willis, P. A. (2017). Enhanced resolution of chiral amino acids with capillary electrophoresis for biosignature detection in extraterrestrial samples. *Analytical chemistry*, 89(2), 1329-1337

However, compared to previous publications, here the authors consider many different samples and clearly provide new results as they also account for the influence of degradation process.

REPLY: Thank you for bringing this work to our attention. We incorporated it into the manuscript and added a new paragraph starting at line 68.

3. Data and methodology: I would highlight more the diversity of samples used in the study and explain more in detail types of samples considered (e.g., Table 1 should be more at the beginning).

REPLY: Thank you for bringing this ambiguity to our attention. We have clarified the scope and diversity of the dataset in the first paragraph of the "Amino acids" subsection in the "Mapping sample dissimilarities across origins" section. Table 1 was moved to the bottom of the paper for clarity (especially now that over 30 new samples have been added to the AA dataset), to avoid interrupting the main text. Table 1 spans two pages. Additionally, we added a per-sample label that may help orient the reader before considering the table. The description of these new labels is in a new paragraph at line 120.

4. Appropriate use of statistics and treatment of uncertainties: not familiar with.

5. Conclusions: the authors highlight the robustness of their method even when considering degradation, but they should add that it concerns only one degradation process (radiolytic degradation).

REPLY: We thank the reviewer for this comment. We model the radiolysis process because it has been shown to be the dominant process on irradiated icy moon surfaces (e.g., Pavlov et al. (2024), Yoffe et al. (2025)). In the paragraph starting at line 313, we made it clear that we modeled only one degradation process (radiolytic degradation). We defer a comprehensive analysis of other degradation processes (e.g., photolysis) across different astrobiological environments to future work.

6. Suggested improvements:

- Include other factors of degradation might improve robustness of the technique.
- Emphasize more the impact of microbial metabolisms: not clear for me if the samples considered as “biotic” in this study include both microbial cells themselves and samples from the surrounding environment of microorganisms (as microbial activity might change the composition of aa around). Check: Schwendner, P., Riedo, A., Melton, D. J., Horvath, P., Lindner, R., Ehrenfreund, P., ... & Cockell, C. S. (2022). Microbial metabolism of amino acids—biologically induced removal of glycine and the resulting fingerprint as a potential biosignature. *Frontiers in Astronomy and Space Sciences*, 9, 781542
- Related to previous comment: if not already done, use cells of *E. coli*, *A. tumefaciens*, *C. butyricum* and *S. marscens* for the analysis of aa composition, as they did for fatty acids.
- Include some microorganisms relevant in astrobiology (methanogens, *Deinococcus radiodurans*)

REPLY:

FACTORS OF DEGRADATION: The scope of our paper is to demonstrate that statistics can clearly differentiate between abiotically and biotically produced amino acid and fatty acid assemblages. In a test case to demonstrate that this also works in a planetary context, we modeled radiolytic degradation and found that our method still produces robust results. Note that we added 15 amino acids (in addition to the 3 previously used), allowing us to apply the radiolytic degradation model to a larger dataset and obtain more robust results. Modeling other degradation processes is beyond the scope of the paper. Moreover, modeling other degradation processes, such as photolysis, is considerably more difficult due to the lack of photolytic constants for many amino acids. Nevertheless, Yoffe et al. (2025) showed that photolytic degradation in Europa-like ice is relevant within the uppermost millimeter of ice. On Mars, the main driver of degradation is likely post-depositional diagenesis, which is considerably more difficult to predict/model.

SAMPLES: The biotic amino-acid assemblages considered here are not restricted to isolated intracellular compositions, but represent environmentally expressed distributions shaped by biological activity. In these systems, amino-acid profiles reflect a combination of cellular material, extracellular products, metabolic processing, and early diagenetic filtering, rather than intact biomass alone.

Within this context, we have added the microbial samples requested by the reviewer, including *Escherichia coli*, *Methanothermobacter thermophila*, *Geobacillus* sp. LC300, *Thermus thermophilus*, *Rhodothermus marinus*, *Methanococcus maripaludis*, *Synechococcus* sp. PCC 7002, and *Alicyclobacillus acidocaldarius*, as listed in the updated table and drawn from published MIC datasets. *Deinococcus radiodurans* was not included because no dry whole-cell biomass amino acid composition is available; existing measurements are limited to intracellular extracts dominated by glutamate and aspartate, which are not representative of the bulk cellular composition and would artificially suppress diversity. Importantly, selective metabolic removal of amino acids, such as glycine, would increase evenness and thereby strengthen the biotic signal, whereas glycine- and alanine-dominated assemblages are characteristic of abiotic synthesis.

MICROBIAL METABOLISM: The biotic samples considered here represent cellular amino-acid compositions rather than surrounding environmental matrices. This choice is conservative: biologically induced removal of amino acids such as glycine is known to imprint strong compositional fingerprints (Schwendner et al. (2022); mentioned now in the paragraph starting at line 135) and, within our framework, would *increase* evenness and thus strengthen the ecodiversity signal. By contrast, glycine- and alanine-dominated assemblages are characteristically abundant in abiotic synthesis and drive sparsity. We have expanded the analysis to include additional bacterial taxa and astrobiologically relevant microorganisms where whole-cell biomass data are available

7. References: include previous works done (see point 2 above). As the authors mention (homo)chirality as a possible biosignature, they should also cite some papers working on this (e.g., Patty, C. L., Ten Kate, I. L., Sparks, W. B., & Snik, F. (2018). Remote sensing of homochirality: a proxy for the detection of extraterrestrial life. In *Chiral Analysis* (pp. 29-69). Elsevier.)

REPLY: Thank you for this suggestion. We have incorporated this reference in the paragraph starting at line 68.

8. Clarity and context:

- Give more context about the degradation's influence (is it only radiolytic degradation that matters? Any other?).
- For the people that are not familiar with this domain, the introduction should better explain the term ecodiversity and how they consider richness and evenness. (super well described in the method section).

REPLY:

DEGRADATION: We thank the reviewer for this comment. We explicitly stated that we consider only radiolysis, as it is the dominant driver of degradation on Europa's surface (Nordheim et al., 2018; Yoffe et al., 2025). Luckily, it is also the best-constrained process and was addressed in our previous work. We noted this in the Discussion section.

INTRODUCTION TO DIVERSITY: Indeed, we put much of the weight on explaining and quantifying the diversity schema in the Methods, but we have updated the paragraph starting at line 92 to provide a better intuition. We hope that this addresses the reviewer's request.

Reviewer #2 (Comments for the Author):

1. Summary of the key results

This study proposes the statistical structure of molecules in a sample as a class of biomarkers for differentiating between abiotic and biotic organics in extraterrestrial samples. The authors applied ecodiversity statistics to organics, instead of species, measuring richness (number of individual molecules) and diversity (uniformity of their relative abundances), then using this information to create an evenness curve (distributional structure for comparing samples with different richness). Data was collected from published studies and includes the number and abundance of individual amino acids or fatty acids from each sample (34 total samples for amino acids, eight total samples for fatty acids) of biotic, abiotic, or mixed origin. The samples were compared against each other through computation of pairwise distances between evenness curve distributions. The authors report that biotic and abiotic amino acid profiles are differentiable from each other with a k-Nearest-Neighbors classification accuracy of 86- 93%, and that within the biotic and mixed origin samples, dissimilarities between evenness curves show samples grouped into highly diverse, degraded/contaminated, or severely degraded, where the most degraded plot towards abiotic samples. The authors also simulated expected degradation rates for three selected compounds under Europa-like conditions, based on radiolytic constants, to assess whether biogenicity or abiogenicity could be determined after degradation, and found that degraded samples were still differentiable. Biotic amino acids displayed greater internal evenness with more uniform abundances, compared to abiotic samples that are sparse and favor smaller compounds. The same test was applied to fatty acids and the authors report that their statistical approach differentiates biotic and abiotic origin, however, the sample set only includes eight total samples with two synthetic abiotic samples and no natural abiotic samples.

2. Originality and significance

Statistical analysis of richness and uniformity is a clever approach for differentiating biotic versus abiotic organics, and the amino acid results show promise. However, as discussed above, a larger sample set is needed to draw firm conclusions. The fatty acid results are not significant due to the small dataset and lack of natural samples. The radiation results also require more data;

including a greater number of molecules beyond the three modeled would be helpful, and any laboratory experiment data from published studies would also be a good addition. The authors also fail to mention or cite the work that has been done with molecular approaches for differentiating biotic from abiotic organics, which is essential to contextualize their results and provide justification for why their approach is ideal (references listed in the “References” segment below).

3. Data & methodology

The authors’ approach is valid and promising for amino acids, but data and methodology for the fatty acid and radiation parts are lacking. In general, more samples are needed, and since calculation of molecular profiles leverages data that is already published and doesn’t require physical analysis of samples, expanding the dataset is a reasonable ask. For the radiation part, the ecosystem diversity metrics do not seem applicable because only three select molecules were modeled, whereas the amino and fatty acid calculations use full suites of molecules, which is key for ascertaining diversity versus richness.

REPLY: We thank the reviewer and agree with their assessment. We expanded the datasets and revised the radiolysis analysis as detailed below.

AMINO ACIDS: In response, we added 32 additional amino acid samples spanning both biotic and abiotic material. On the abiotic side, we added 24 chondrite samples across multiple groups and alteration states, including pristine and potentially contaminated samples. On the biotic side, we added 8 fresh biomass samples, including astrobiologically relevant microbes such as methanogens (Table 1). The amino-acid dataset, therefore, increased to 69 samples (from 37), and the separation between biotic and abiotic profiles strengthened.

FATTY ACIDS: We expanded the fatty-acid dataset to 28 samples (from 8), adding meteoritic and Fischer–Tropsch-type abiotic distributions, additional biotic profiles, and geologic lipid assemblages drawn from multiple studies spanning soils and sediment cores across different depositional regimes and burial depths. Because published biotic and abiotic studies often report disjoint chain-length ranges (biotic typically $C > 12$, abiotic typically shorter), we restrict the analysis to straight-chain species and to the overlapping window where coverage is sufficient for a like-for-like comparison (abiotic: $C > 7$). Within these constraints, the biotic–abiotic distinction persists and is quantified using the same kNN framework applied to amino acids, with significance assessed by bootstrapping and permutation tests (new Figures 2 and 4; Extended Data). Given the remaining coverage limitations, we present the fatty-acid analysis as a proof-of-concept secondary result that complements the amino-acid analysis.

RADIOLYSIS: We expanded the radiolysis analysis to include radiolytic constants for 15 amino-acid species embedded in biological material in ice (Pavlov et al., 2024), and we include only samples in our dataset that contain at least 7 of these species. The degradation analysis is therefore more robust and now samples biotic and abiotic sample pairs while propagating uncertainty by drawing per-species radiolytic constants within their reported ranges. These results indicate stronger persistence of the diversity signal under Europa-like radiolytic processing, and we again thank the reviewer for prompting this expansion.

4. Appropriate use of statistics and treatment of uncertainties

The ecodiversity approach to analysis of molecular data for amino and fatty acids shows promise and is unique in its ability to capture diversity and richness in one metric. The statistical tests (e.g., Mann-Whitney U, KS, AUC, Kolmogorov-Smirnov, functional ANOVA) discussed in the supplemental are applied appropriately.

REPLY: We thank the reviewer for their comment.

5. Conclusions: robustness, validity, reliability

The authors’ results from amino acid analyses provide a compelling argument for evenness as a potential indicator of biogenicity or abiogenicity, and I agree that this could be a promising detector-agnostic metric for assessing diversity and richness. However, I would have liked to see a lot more samples, considering the input data for each individual sample was a discrete list of molecular abundances collected from published literature, so there is plenty more to pull from and doesn’t require additional experimental analyses. I would especially have liked to see more data in the abiotic category – even within the selected studies there are several other meteorite samples with amino acid data reported, and it’s unclear why the authors chose to exclude those

ones. On the biotic side, I find it intriguing that the severely altered samples plot towards abiotic, which reaffirms expectations that diagnosticity is lost with processing. I enjoyed the sample-by-sample discussion of outliers in the “Resolving Biotic Sample Histories” section, and think that a larger sample suite that includes more modern or fresh material could help deconvolve the trends and outliers further, especially if those samples are dominated by microbial life.

While the amino acid analyses are promising, I do not find the fatty acid analysis to be publication-worthy at this stage. Eight total samples with two synthetic abiotic samples is too small of a dataset to draw conclusions from, especially considering that both abiotic samples are synthetic. Of course, indigenous long-chain fatty acids are not present in meteorites, but shorter-chained species C1- C12 are, and the authors could pull data from these studies. This sort of test is needed to validate the model’s efficacy, since this type of statistical analysis is blind to the identity of individual species within one type of compound class, and carboxylic/fatty acids with differing chain lengths are present in both biotic and abiotic natural samples. For astrobiological relevance, a reference that the authors may want to consult is Deamer et al. (1986), who showed that carboxylic acids C8+ extracted from meteorites form protomembrane-like structures. For the biotic fatty acids, the authors should include data from geologic samples instead of just individual organisms, because mixed input and varying states of degradation yield different profiles and are much more astrobiologically relevant.

I agree that it’s important to consider how the effects of radiation could skew a sample’s organic distribution, but more data is needed before the authors can conclude that biotic and abiotic will be differentiable, beyond modeling of three selected amino acids. The ecodiversity statistical approach does not seem applicable if only three compounds are sub-selected, since an overall view of richness versus diversity is required to make that assessment. I was also confused by the “Model Description” section in the supplemental, because as written, the authors applied radiolytic decay models to every single biotic sample in the dataset, and I’m unclear on how this translates to Figure 8 in the supplemental and Figure 5 in the main text.

REPLY: We have addressed all of the reviewers’ remarks made here in Section 3 (Data & Methodology), in particular:

SAMPLES: We are glad that the reviewer enjoyed our sample-by-sample discussion. As requested by the reviewer, we added 32 amino acid samples and 20 fatty acid samples, covering both abiotic and biotic samples. Please note that we did not intentionally exclude any samples, except for those analyzed across multiple studies, in which case we consider the most recent analysis for each sample (e.g., Orgueil, Murchison). More details about the newly added samples are explained above.

FATTY ACIDS: As explained above, our dataset now comprises 28 fatty acid samples, including several meteorites, geologic samples, and organisms (Extended Data Table 2). We have justified our decision to consider relatively long-chain fatty acids (≥ 7) in our analysis by citing the observation of Deamer et al. (1986).

RADIOLYTIC DEGRADATION MODEL: We thank the reviewer for this comment. The radiolytic decay model is applied to all eligible biotic samples, excluding those classified as Biotic Degraded, to avoid conflating pre-existing diagenetic overprinting with radiolytic processing, as described in the Extended Data. We further restrict the analysis to samples containing at least 7 amino acid species for which radiolytic decay constants (from Pavlov et al., 2024) are available. Figure 8 presents the depth- and location-dependent radiation dose rates on Europa used in the model (from Yoffe et al., 2025), while Figure 5 summarizes the resulting ensemble behavior. In each realization, one biotic and one abiotic sample are randomly drawn, propagated under identical radiolytic conditions using species-specific decay constants sampled within their uncertainties, and converted to evenness curves through time. Figure 5, therefore, reports ensemble-averaged dissimilarities and variability across many realizations, rather than tracking individual sample trajectories.

6. Suggested improvements

As mentioned in previous comments, the work would greatly benefit from more samples. On the abiotic amino acid side, it would have been useful for the authors to note the meteorite’s group (CI, CM, CO, CV, CK, CR, CH, CB), because amino acid content can vary across these categories. The authors should also consider that Bennu and Ryugu are both CI-type bodies, which are both unique and rare amongst meteorites (Orgueil being an exception), but they are also returned asteroid samples, so the difference in organic content could be due to the friable nature of this material and/or alteration that occurs during meteoritic atmospheric entry. For the biotic amino acid side, modern samples and samples that are dominated by microbial input are crucial additions, especially to support the application to Europa, where simple extant life is a consideration. The radiation part should be

re-thought; as mentioned in my comments under the “Data and Methodology” segment, selecting three amino acids and applying degradation models does not fit with the ecosystem diversity statistical approach because it inherently eliminates the ability to accurately measure both diversity and richness.

REPLY: Our replies to the reviewer’s specific comments are listed below.

LABELS: We have added labels for both biotic and abiotic samples (including meteorite types) to better orient the reader, as suggested.

SAMPLES: We have added the following samples dominated by microbial input: *Escherichia coli*, *Methanothermobacter thermophila*, *Geobacillus* sp. LC300, *Thermus thermophilus*, *Rhodothermus marinus*, *Methanococcus maripaludis*, *Synechococcus* sp. PCC 7002, and *Alicyclobacillus acidocaldarius*. These now form the most diverse edge of the diversity scale established in our dataset, whereas on the sparsest end are chondrites that were relatively unaltered aqueously (e.g., ureilites, CO3, CV3, Benu).

RADIOLYSIS: We have addressed the reviewer’s concern about the radiolysis model in Sections 3 and 5 of this point-to-point response.

7. References

In lines 36- 38, Napier, W. A (2004) is incorrectly cited – this paper discusses panspermia, or the concept that organisms that originated on one body could be transferred to another body, where they could be reanimated and form the basis for an independent ecosystem. The language, “widespread presence of organic molecules in extraterrestrial bodies... suggest[s] life’s precursors arrived via meteorites and comets” is not panspermia. Instead, cite one of the following:

Pizzarello, S. (2006). The chemistry of life’s origin: a carbonaceous meteorite perspective. *Accounts of Chemical Research*, 39(4), 231-237.

Osinski, G. R., Cockell, C. S., Pontefract, A., & Sapers, H. M. (2020). The role of meteorite impacts in the origin of life. *Astrobiology*, 20(9), 1121-1149.

Sandford, S. A., Nuevo, M., Bera, P. P., & Lee, T. J. (2020). Prebiotic astrochemistry and the formation of molecules of astrobiological interest in interstellar clouds and protostellar disks. *Chemical reviews*, 120(11), 4616-4659.

Deamer, D., Dworkin, J. P., Sandford, S. A., Bernstein, M. P., & Allamandola, L. J. (2002). The first cell membranes. *Astrobiology*, 2(4), 371-381.

REPLY: Thank you. Corrected. We now cite Deamer et al. (2002).

The authors do not reference any of the published work on molecular approaches for differentiating between biotic and abiotic organics. A subset of this body of work is listed below:

Teece, B. L., Beaty, D. W., Graham, H. V., McDonnell, G., Sherwood Lollar, B., Siljeström, S., ... & Mackelprang, R. (2025). The abiotic background as a central component of a sample safety assessment protocol for sample return.

Buckner, D. K., Anderson, M. J., Wisnosky, S., Alvarado, W., Nuevo, M., Williams, A. J., ... & Wilhelm, M. B. (2024). Quantifying Global Origin-Diagnostic Features and Patterns in Biotic and Abiotic Acyclic Lipids for Life Detection. *Astrobiology*, 24(1), 1-35.

Dorn, E. D., Neelson, K. H., & Adami, C. (2011). Monomer abundance distribution patterns as a universal biosignature: examples from terrestrial and digital life. *Journal of molecular evolution*, 72(3), 283-295.

Saeedi, D., Buckner, D., Walton, T. A., Aponte, J. C., & Aghazadeh, A. (2025). Discriminating abiotic and biotic organics in meteorite and terrestrial samples using machine learning on mass spectrometry data. *PNAS nexus*, 4(11), pgaf334.

Johnson, S. S., Anslyn, E. V., Graham, H. V., Mahaffy, P. R., & Ellington, A. D. (2018). Fingerprinting non-terran biosignatures. *Astrobiology*, 18(7), 915-922.

Sherwood Lollar, B., Westgate, T. D., Ward, J. A., Slater, G. F., & Lacrampe-Couloume, G. (2002). Abiogenic formation of alkanes in the Earth's crust as a minor source for global hydrocarbon reservoirs. *Nature*, 416(6880), 522-524.

Cleaves, H. J., Hystad, G., Prabhu, A., Wong, M. L., Cody, G. D., Economon, S., & Hazen, R. M. (2023). A robust, agnostic molecular biosignature based on machine learning. *Proceedings of the National Academy of Sciences*, 120(41), e2307149120.

Mißbach, H., Schmidt, B. C., Duda, J. P., Lünsdorf, N. K., Goetz, W., & Thiel, V. (2018). Assessing the diversity of lipids formed via Fischer-Tropsch-type reactions. *Organic Geochemistry*, 119, 110-121.

Glavin, D. P., Burton, A. S., Elsila, J. E., Aponte, J. C., & Dworkin, J. P. (2019). The search for chiral asymmetry as a potential biosignature in our solar system. *Chemical reviews*, 120(11), 4660-4689.

REPLY: Cleaves+23 is cited (line 75), in addition to Mißbach+18 (new citation and fatty-acid sample). In addition, we have added several citations, as suggested by Reviewer #1. These are:

- McDonald, G. D., & Storrie-Lombardi, M. C. (2006). Amino acid distribution in meteorites: diagenesis, extraction methods, and standard metrics in the search for extraterrestrial biosignatures. *Astrobiology*, 6(1), 17-33.

- Creamer, J. S., Mora, M. F., & Willis, P. A. (2017). Enhanced resolution of chiral amino acids with capillary electrophoresis for biosignature detection in extraterrestrial samples. *Analytical chemistry*, 89(2), 1329-1337

We have also cited Buckner et al. (2024) in the introduction (line 71).

8. Clarity and context

Reviewer #2 (Remarks on code availability):

I am able to access the site and view the data in CSV format, but I do not have the expertise to run the code myself.

REPLY: We will provide more digestible and easily executable versions of our analyses for amino acid, fatty acid, and radiolytic degradation modeling, and upload them to Zenodo.

NATASTRON-25070921B Rev-2 Reply to Reviewers

We thank the reviewers for their careful evaluation of the revised manuscript and for confirming that the revisions addressed their suggestions. Below, we respond to the remaining comments. In accordance with Nature Astronomy's editorial requirements, the manuscript has been slightly shortened to comply with the journal's length limits. Text explicitly added or modified in response to the reviewers is highlighted in bold.

Reviewer #1 (Comments for the Author):

Comments for the Author:

I thank the authors for taking into account all my suggestions and include them in their paper. For me, now the article is much easier to read/understand and can definitely be published in the journal Nature astronomy.

REPLY: We thank the reviewer for their comments and positive feedback! We are glad that the embedded suggestions were done as the reviewer suggested.

Here are some minor remarks:

3. Data and methodology:

Much clearer like this. Thanks for adding the small paragraph at the beginning of amino acid subsection. I understand that it might make the table 1 even longer, but maybe it would be good to include the per-sample label as well in this table?

REPLY: We thank the reviewer for this suggestion. We have added the sample labels to all tables

7. References:

Not sure about where you included the reference about the work done by Patty et al. It is with the line talking about homochirality itself, but not the method we can use to detect it (circular polarization)..

REPLY: Patty et al. (2018) is cited in the context of using chirality as a biosignature at line 47.

Reviewer #2 (Comments for the Author):

2. Originality and significance: if not novel, please include reference

I appreciate the authors' expansion of their datasets, and agree that that the results are original and significant. I also appreciate the added references to previous work differentiating biotic from abiotic organics. However, I would like to see a little more text discussing the authors' results within the context of that body of literature. I recognize space is limited, so even a short paragraph in the discussion section would do. My reasoning is that I believe this will strengthen the applicability of their approach and properly contextualize the results – previous papers have demonstrated that biotic and abiotic organics differ, and the unique methodology in this work reiterates those previous findings while providing a complementary approach that does not rely on identification of specific molecular structures.

REPLY: We thank the reviewer for this helpful suggestion. We agree that the original manuscript did not sufficiently place the diversity-based framework within the broader literature on distinguishing biotic from abiotic organic assemblages. To address this, we added two short paragraphs in the Discussion (lines 300 and 306) that more explicitly relate our results to prior approaches based on molecular identity, chirality, isotopic composition, and distribution-level metrics. These additions clarify that the diversity framework complements existing methods by capturing a statistical property of molecular assemblages that does not depend on identifying specific compounds.

3. Data & methodology: validity of approach, quality of data, quality of presentation

The additional samples and analyses that the authors have included have provided added value and enhanced the robustness of the results. Regarding the fatty acids, I wonder why branch chained compounds were excluded. I understand the argument that straight chained compounds are preferred by biology for membrane formation, but cyclic and unsaturated fatty acids were included within the biotic set, and many microbes synthesize methyl branched fatty acids for similar structural enhancements. A driving argument for the statistical approach is that identification of individual molecular structures is not required, so including branched compounds is important, especially because the amino acid analysis included all isomers identified within a sample set, regardless of similarities in molecular weight. The authors mention that differing abiotic fatty acid reaction pathways produce straight versus branched compounds, but natural samples can contain input from multiple sources, so adding this tweak would make the results even more applicable to analysis of samples from other planetary bodies.

REPLY: We thank the reviewer for this insightful comment. Following this suggestion, we expanded the fatty-acid analysis to include branched compounds. In doing so, we found that meteoritic fatty acids separate into two internally coherent populations—straight-chain and branched isomers—with distinct evenness patterns. In the main analysis (Figure 3), straight and branched chains from each sample are therefore analyzed separately and plotted as distinct assemblages, which preserve their internal diversity structure and maintain clear separation from biotic samples. However, these two subsets differ substantially in absolute abundance, and when pooled into a single assemblage, this imbalance dominates the distribution and obscures the diversity signal. As discussed in the literature we incorporated, straight- and branched-meteoritic fatty acids may also reflect partially decoupled formation and alteration pathways. We note this observation in the main text (a new paragraph starting at line 201) and, for completeness, we show and discuss the pooled case in the Supplementary Information, where the effect of combining both subsets on the diversity signal is illustrated.

6. Suggested improvements: experiments, data for possible revision

I only have a few minor suggestions, as I feel the authors have done a great job of expanding their analyses from my previous comments.

- As mentioned in the “Originality and significance” section, I would appreciate contextualization of their results with a short discussion of past work with traditional molecular methods. In my opinion, this addition would provide an enhanced argument for organics-based life detection efforts because it demonstrates that this type of biosignature contains a great deal of information. For astrobiology mission applications, this can help demonstrate that organic analyses are crucial, because biotic and abiotic compounds can be differentiated through either molecular identification, statistical approaches, or both.

- As mentioned in the “Data & methodology” section, I would like to see branched fatty acids included. Hopefully this will not be too heavy of a lift!

- I recommend a different color scheme for Figure 5 – it’s hard to see the difference between “biotic” and “degrading biotic.”

- The caption for Figure 3 could use more information. Alternatively, the authors could combine Figure 2 and 3 to avoid repeating text.

- This is very minor, but the term “panspermia” in the introduction section needs to be removed. Panspermia is the idea that viable organisms were transported from one planetary body to another, not that molecules delivered exogenously contributed to an independent origin of life event.

REPLY:

We thank the reviewer for these helpful suggestions and for their positive assessment of the revisions. We address each point below.

Contextualization with previous work.

As suggested, we added a short discussion placing our results within the context of previous work that distinguishes biotic and abiotic organics using traditional molecular approaches. This addition appears in the Discussion (lines 266) and highlights how

the diversity-based framework complements methods based on molecular identity, chirality, isotopic composition, and targeted distributional patterns.

Inclusion of branched fatty acids.

Following the reviewer's suggestion, we expanded the fatty-acid analysis to include branched compounds. Straight and branched fatty acids are analyzed disjointly in the main analysis (Figure 3), where they are plotted as separate assemblages for each sample. This preserves their internal diversity structure and maintains clear separation from biotic samples. In the Supplementary Information, we also show and discuss the effect of pooling straight and branched compounds into a single assemblage.

Figure 5 color scheme.

We agree that the original color scheme reduced the visual distinction between curves. The color scheme in Figure 5 has been revised to improve clarity and better differentiate the biotic and degrading biotic trajectories.

Figure 3 caption.

We revised the caption for Figure 3 and added additional explanatory text to clarify the analysis and sample grouping. These changes improve readability and reduce redundancy with the preceding figure.

Use of the term "panspermia."

We thank the reviewer for noting this. The term has been removed from the Introduction, and the wording has been corrected to refer more precisely to exogenous delivery of organic molecules rather than the transfer of viable organisms.