Web links to the author's journal account have been redacted from the decision letters as indicated to maintain confidentiality.

28th Apr 21

Dear Dr. Wagner,

Your manuscript titled "Cyanate – a low abundant but actively cycled nitrogen compound in soil" has now been seen by our reviewers, whose comments appear below. In light of their advice I am delighted to say that we are happy, in principle, to publish a suitably revised version in Communications Earth & Environment under the open access CC BY license (Creative Commons Attribution v4.0 International License).

We therefore invite you to revise your paper one last time to address the remaining concerns of our reviewers. In particular, we ask that you provide a balanced discussion of the caveats and limitations of your study, and appropriately tone down some statements. At the same time we ask that you edit your manuscript to comply with our format requirements and to maximise the accessibility and therefore the impact of your work.

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Please review our specific editorial comments and requests regarding your manuscript in the attached "Editorial Requests Table". Please outline your response to each request in the right hand column. Please upload the completed table with your manuscript files.

If you have any questions or concerns about any of our requests, please do not hesitate to contact me.

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We hope to hear from you within two weeks; please let us know if you need more time.

Best regards,

Clare

Dr Clare Davis
Associate Editor
Communications Earth & Environment

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REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

Review of the manuscript “Cyanate – a low abundant but actively cycled nitrogen compound in soil“ by Mooshammer et al., submitted to Communications Earth & Environment in March 2021
In this paper, cyanate concentrations in mineral topsoils from 42 sites along a latitudinal gradient from south France to north Norway are presented along with two experiments to determine cyanate turnover in soil and cyanate formation from urea. Additionally, cyanate concentrations were determined in salt marshes, pore water of salt marshes, activated waste water sludge and discharge of waste water treatment plants. These own data were amended with literature values of cyanate concentrations in ocean water.
The authors demonstrate that cyanate occurs in many soils at low concentrations and is microbially turned over in soils at high rates. The major claim stated already in the title is well supported.
The results of this study are novel and interesting to the community, because they contribute to completing the picture of the N cycle in terrestrial ecosystems.
The presented field and experimental data are comprehensive and thus the whole paper convincing.
The paper mainly helps in elucidating the minor and up to now neglected N cycling processes, which is valuable and exciting, but I don’t expect that it will strongly influence the thinking in the field or change the state-of-the-art N cycling conceptual and mathematical models. Another advancement of the field consists of the reported method adaptation to determine cyanate concentrations and

even stable C and N isotope ratios in cyanate extracted from soil, a chemically complex and technically difficult environmental matrix.
Overall, the paper is well written. I have only a number of minor suggestions to further improve it.
l. 27: Replace “relatively low” by a quantitative measure such as the range of ammonium/cyanate ratios reported later or a percentage of inorganic or total N concentrations to give the reader an impression of the importance of cyanate.
l. 29: Add “in unfertilized soils”
l. 33-34: I think that this is too far stretched. You cannot support an evolutional advantage of the use of cyanate with your work (and even didn’t intend to do this). Given the species that are able to process cyanate, I have more the impression that the capability of processing cyanate is a remnant of a time when cyanate might have played a more important role. – But this is similarly a subjective speculation of myself. I would nevertheless tone down this conclusion (e.g., perhaps leave it in the discussion but remove it from the abstract and conclusions).
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l. 51: As far as I know, plants can only assimilate ammonium via the glutamate synthetase, not ammonia.
l. 62 (and in the whole paragraph): Spell genus and species names in italics.
l. 72-73: “ammonium” instead of “ammonia” (2x)?
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l. 117: This interpretation depends on the reason for the low cyanate recovery in acid soils. If the low recovery was attributable to cyanate turnover, you were right, if it was attributable to non-extractable sorption, you were not right. Again, an earlier discussion of the cyanate turnover and sorption processes would help.
l. 138: Alternatively, cyanate could be strongly adsorbed (e.g., as inner-spheric complex) to Fe and Al oxides. You mention this possibility later, but should discuss it here (l. 152).
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l. 290, 302 and throughout the manuscript: Spell small numbers out, i.e.. “three”?
l. 301: Add “In unfertilized soils, urea concentrations…”
l. 324-328: This section repeats numbers shown in Fig. 5, which is not necessary. Delete.
l. 343-347: This is not a conclusion but rather belongs to the discussion.
l. 350: “relatively” to what?
l. 354: This is unsupported speculation.
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l. 388: Perhaps add “in field-fresh state”, because usually soils are dried before sieving and add sampling depth of the rice paddy soil.
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l. 404 and 405: Usually plant names are accompanied by author abbreviations, when first mentioned.

l. 410-412: For what did you use Rhizon samplers? I have missed this. Delete? The soil solution of the marshes was won by centrifugation.
l. 443 and 445-446: Replace “content” by “concentration” and give C and N concentrations in the SI units mg g-1 (not %).
l. 445: Give unit as mS m-1 to be consistent with the other units.
l. 449: How was WHC determined?
l. 466: In my experience, derivatization is a critical step with respect to stable isotope ratio determination. Please add the degree of derivatization you have reached. I think that you can only accept a loss of ca. 5% to rule out a methodological bias.
l. 495: Did you check the stability of cyanate during your rather long analysis procedure?
l. 549: “nmol L-1”.
l. 596: Mention how you tested the prerequisites for t-tests and ANOVA (i.e., homoscedasticity and normal distribution of residuals).
l. 781: Replace 0.01 M by 10 nM CaCl2 for consistency with the text.
l. 768: Do not include “redrawn from” in the superscript. And shouldn’t it be “redrawn from ref. 49” or “from name of author(s)” and 49 as superscript?
Fig. 4: There are two subfigures “D”. Please explain the meaning of the lines in subfigures B-D.

Reviewer #2 (Remarks to the Author):

The manuscript (Cyanate – low abundant but actively cycled nitrogen compound in soil) by Mooshammer et al. attempts to describe cyanate cycling in terrestrial systems. Overall, I found the description and experiments very compelling and am intrigued by the role that this process may play in many different ecosystems. The relative paucity of other reports on this phenomenon in terrestrial systems combined with the potential impacts this could have on broader biogeochemical cycles makes this a study that will likely be interesting to a wide range of readers. I can point out only a few (relatively minor) points for potential clarification as listed below:

• If I understand correctly, the isotope tracer experiments are used to estimate the cyanate turnover rates in soil. Lines 455-457 indicate that the cyanate added to soil was approximately 250 times the typical concentrations. Given this, how does the reported biotic half life of cyanate (generated from tracking the decay of a relatively large burst of added material) reflect turnover at more natural concentrations?

• The isotope tracer experiment was very interesting and I appreciate the mention/description of the method used for this. In addition to tracking the loss of cyanate, was this approach able to identify any other fates of the 15N-labeled cyanate? Or, could other approaches be applied in the future to determine whether any of this nitrogen was entering into biomass pools (microbial, plant or otherwise)? The discussion in lines 254-262 highlight potential uptake into microbial biomass but any tracking of the 15N tracer into the biomass pool would be extremely compelling if this were available.
o Similarly, were water extracts or other fractions from the samples that may contain microbial

metabolites considered for analysis via an Orbitrap method for identification of 15N containing compounds?
• Lines 380-381: There is mention of samples being stored at -80 deg C as there is less decay of material over time. Can the authors comment on typical storage times between sample collection and analysis?

• Figure 5: The authors identified a marked distinction between NH4+/NCO- typical of terrestrial versus marine settings. Can the authors speculate on why this may occur? Is this driven by the low turnover of cyanate the authors mention (lines 337-340) and, if so, any potential reasons for the low turnover rate in marine settings versus terrestrial?

• A couple potential typos to consider:
o Line 131: presumable the authors intend "…/ammonium and carbon dioxide/…" (instead of "and on dioxide"
o Figure 4: Has two panels labeled "D" and no panel "E" in the figure (but E is listed in the caption)

Reviewer #3 (Remarks to the Author):

Authors of this work investigate cyanate availability and dynamics in soils, providing evidence that cyanate is actively (and mainly) cycled by biotic processes. The work is original and makes a significant contribution to the understanding of the environmental role and significance of cyanate in terrestrial ecosystems, a matter hitherto almost entirely unknown. The experimental work was nicely designed and conducted, and the results are robust and of general interest. Therefore, I feel that there is merit to publishing the paper without serious modifications.

Comments:

1. Authors found that consumption of isotopically labelled cyanate was substantially higher in grassland soil than in arable soil, and that this consumption was mainly biotic, with only small contributions from abiotic processes. This result should be discussed more deeply, giving a possible explanation for this. Grassland soils are usually nutrient-rich, with fertile upper soil layers, but urea fertilizers are used in arable lands probably resulting in a significant formation of cyanate.

2. Although microbial biodiversity is not a goal of this work, a metagenomic analysis to stablish the relative abundance and diversity of the microorganisms able to degrade cyanate in the grassland and arable soils could be an interesting complement to this work.

3. Some data and calculations given in Table 1 and discussed in pages 11-12 are estimations that should be taken with cautions. I recommend considering and discussing these data more prudently.

4. In the titles of the cited references, the scientific names of the organisms should be written in italics.

RESPONSE TO REVIEWERS' COMMENTS:

We very much appreciate the constructive and helpful assessment of our manuscript by the reviewers and editor. We carefully revised the manuscript, and below you find our point-by-point response to all points raised during review.

Reviewer #1 (Remarks to the Author):

Review of the manuscript "Cyanate – a low abundant but actively cycled nitrogen compound in soil" by Mooshammer et al., submitted to Communications Earth & Environment in March 2021
In this paper, cyanate concentrations in mineral topsoils from 42 sites along a latitudinal gradient from south France to north Norway are presented along with two experiments to determine cyanate turnover in soil and cyanate formation from urea. Additionally, cyanate concentrations were determined in salt marshes, pore water of salt marshes, activated waste water sludge and discharge of waste water treatment plants. These own data were amended with literature values of cyanate concentrations in ocean water.
The authors demonstrate that cyanate occurs in many soils at low concentrations and is microbially turned over in soils at high rates. The major claim stated already in the title is well supported.
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The paper mainly helps in elucidating the minor and up to now neglected N cycling processes, which is valuable and exciting, but I don't expect that it will strongly influence the thinking in the field or change the state-of-the-art N cycling conceptual and mathematical models.
Another advancement of the field consists of the reported method adaptation to determine cyanate concentrations and even stable C and N isotope ratios in cyanate extracted from soil, a chemically complex and technically difficult environmental matrix.
Overall, the paper is well written. I have only a number of minor suggestions to further improve it.

Thank you very much for your positive feedback.

l. 27: Replace "relatively low" by a quantitative measure such as the range of ammonium/cyanate ratios reported later or a percentage of inorganic or total N concentrations to give the reader an impression of the importance of cyanate.

Done. We have amended the abstract as follows: "Soil cyanate concentrations were three orders of magnitude lower than ammonium or nitrate.".

l. 29: Add "in unfertilized soils"

We state in the abstract "…cyanate was produced *in-situ* at rates similar to that of cyanate formation from urea fertilizer decomposition". Thus, we feel that adding "in unfertilized soils" would be sort of redundant.

l. 33-34: I think that this is too far stretched. You cannot support an evolutional advantage of the use of cyanate with your work (and even didn't intend to do this). Given the species that

are able to process cyanate, I have more the impression that the capability of processing cyanate is a remnant of a time when cyanate might have played a more important role. – But this is similarly a subjective speculation of myself. I would nevertheless tone down this conclusion (e.g., perhaps leave it in the discussion but remove it from the abstract and conclusions).

We removed it from the abstract.

l. 44: I think that this should be “ammonium”, not ammonia – please check throughout the manuscript for correct use of “ammonia” (NH3) and “ammonium” (NH4+).

Thank you. We corrected it and carefully checked the manuscript regarding the use of ammonia vs. ammonium.

l. 51: As far as I know, plants can only assimilate ammonium via the glutamate synthetase, not ammonia.

We now write: The resulting ammonia (which is in equilibrium with ammonium) and carbon dioxide can then be assimilated

l. 62 (and in the whole paragraph): Spell genus and species names in italics.

In this paragraph we refer to organisms that are uncultured. The correct nomenclature is *Candidatus* or short *Ca*. (in italics) followed by the name in Roman type.

l. 72-73: “ammonium” instead of “ammonia” (2x)?

We respectfully disagree. The product of the reaction is ammonia and not ammonium and all data available today point to ammonia as the substrate for ammonia oxidizing microorganisms.

l. 108: At this point, I expected an explanation of the low recovery (turnover/sorption of cyanate), which is only given one page later. Perhaps, you can move the discussion of the cyanate turnover and sorption processes nearer to this finding.

We added an explanation and referred the text that follows. In L106 we added: “The stability of cyanate is pH-dependent, which is explained in more detail below.”

l. 117: This interpretation depends on the reason for the low cyanate recovery in acid soils. If the low recovery was attributable to cyanate turnover, you were right, if it was attributable to non-extractable sorption, you were not right. Again, an earlier discussion of the cyanate turnover and sorption processes would help.

As mentioned in our response to the previous point we now highlight pH-dependent stability of cyanate early in the manuscript and after re-reading this modified section several times, we feel that it now nicely reports our findings and puts them in context with cyanate turnover and sorption processes.

l. 138: Alternatively, cyanate could be strongly adsorbed (e.g., as inner-spheric complex) to Fe and Al oxides. You mention this possibility later, but should discuss it here (l. 152).

In this section, we discuss abiotic reactions that mostly occur at low pH, whereas the reactions discussed in the following paragraph are occurring at neutral to high pH. Thus, discussing

those reactions together as suggested would not be consistent with this structure and we thus would prefer discussing this later.

l. 218: Shouldn't the equations be numbered in the sequence they are called out in the main text?

The equations have the desired order in the Material and Method section. In the main text, we do not present the equations but only refer to selected equations that are given in the Material and Method section.

l. 237-240: This is a repetition from l. 209-214 and can therefore be deleted.

Done.

l. 263-264: This sentence is too complicated. Perhaps just "…how much… is unknown."

Done

l. 266-272: This section is confusing. You start with mentioning that you "used" MRTs of cyanate to calculate gross cyanate production rates but than you "computed" MRTs. Please rewrite. If I understood it correctly, you determined MRTs from the urea addition experiment (with the "flux" being the application rate of urea?) and used this in the other experiment to calculate gross cyanate production rates.

We computed the MRTs of the urea addition experiment in order confirm that we observe similar MRT using two different approaches. To make this clearer, we added to text the following sentence (L271): "Estimating MRTs for both the urea addition and the tracer addition experiment allows to explore whether MRTs are similar using two different experimental approaches.".

l. 290, 302 and throughout the manuscript: Spell small numbers out, i.e.. "three"?

Done.

l. 301: Add "In unfertilized soils, urea concentrations…"

Done.

l. 324-328: This section repeats numbers shown in Fig. 5, which is not necessary. Delete.

We would prefer to keep the mentioning of the results in the text because the data are displayed on a log-scale in Fig. 5, where it is difficult for the reader to quickly catch the mean/median.

l. 343-347: This is not a conclusion but rather belongs to the discussion.

Done. We moved the second sentence of the conclusion to the discussion (L314.)

l. 350: "relatively" to what?

By using the word "relatively" we express that the production rates are high for cyanate, a compound that occurs at a very low concentration, but which does not mean the production rates are as high or higher than, for example, for ammonium and nitrate.

l. 354: This is unsupported speculation.

We respectfully disagree. In the introduction we summarized the findings that show that microorganisms are capable of growing on cyanate as the sole energy source. Our work provides evidence that cyanate occurs in soils, where it is actively produced. In the conclusion we bring this together. In addition, we are careful in the language that we use. We say that "using cyanate **could represent** a selective advantage".

l. 357-363: Again, discussion but not conclusion.

We agree, but we discuss this here to conclude in the next sentence that additional studies of the fate of cyante in soils are required. To keep this flow we would prefer not changing the structure of this paragraph.

l. 388: Perhaps add "in field-fresh state", because usually soils are dried before sieving and add sampling depth of the rice paddy soil.

Added.

l. 399: Did you check the cyanate stability during storage?

We refer to the study by Widner *et al.* (2013), which showed that cyanate was stable at -80°C over a period of 270 days. The storage time of our samples ranged between few days to a maximum of few months. We now mention this in the revised manuscript (L 384).

l. 404 and 405: Usually plant names are accompanied by author abbreviations, when first mentioned.

Added.

l. 410-412: For what did you use Rhizon samplers? I have missed this. Delete? The soil solution of the marshes was won by centrifugation.

For the urea addition experiment, soil solution was obtained by centrifugation, whereas for the pore water extraction from salt marsh sediments we used Rhizon samples. This is described in Materials and Methods.

l. 443 and 445-446: Replace "content" by "concentration" and give C and N concentrations in the SI units mg g-1 (not %).

Done.

l. 445: Give unit as mS m-1 to be consistent with the other units.

Changed.

l. 449: How was WHC determined?

Water holding capacity was determined by saturating the soil and the gravimetric water content was determined after excess water was drained. We added this to the text (L 453).

l. 466: In my experience, derivatization is a critical step with respect to stable isotope ratio

determination. Please add the degree of derivatization you have reached. I think that you can only accept a loss of ca. 5% to rule out a methodological bias.

There is the possibility of isotope fractionation processes associated with derivatization that can cause a deviation in the measured isotope ratio. This is of concern when analysing ^{15}N natural abundance (δ-values) (e.g., Takizawa et al., 2020). In this study, we did not analyse natural abundance of isotopes. We worked with a highly enriched tracer, where fractionation processes associated with the derivatization of the compound are neglectable, which is due to simple mathematical reasons. We used 98-99at% tracer, where the isotope ratio (at%) is $^{13}C/(^{12}C+^{13}C)$. Difference in natural abundance and isotope fractionations usually occur at and beyond the third significant figure of the isotope ratio (Michener & Lajtha, 2008; Hayes, 2004). Therefore, changes in at% are several orders of magnitude larger than any isotope fractionation factor.

We also derivatized concentration standards and isotope standards with each sample batch, which accounts for and corrects for any isotope fractionation due to incomplete yield of derivatized product (L429 and L493). To state clearly in the text that we included those standards, we amended the text as follows: "To improve the accuracy of absolute quantification, external calibration (concentration standards and $^{13}C^{15}N$- KOCN standards) was paired with an internal calibrant (^{13}C-potassium cyanate) to correct for deviations in liquid-liquid extraction efficiency, ionization efficiency and ion suppression."

The primary publication reporting this derivatization procedure stated that the degree of derivatization is > 90% (Widner et al., 2013). We are confident that we have determined the isotope ratio of cyanate as accurately as possible.

Takizawa, Y., Takano, Y., Choi, B., Dharampal, P. S., Steffan, S. A., Ogawa, N. O., ... & Chikaraishi, Y. (2020). A new insight into isotopic fractionation associated with decarboxylation in organisms: implications for amino acid isotope approaches in biogeoscience. Progress in Earth and Planetary Science, 7(1), 1-13.

Michener, R., & Lajtha, K. (Eds.). (2008). Stable isotopes in ecology and environmental science. John Wiley & Sons.

Hayes, J. M. (2004). An introduction to isotopic calculations. Woods Hole Oceanographic Institution, Woods Hole, MA, 2543.

Widner, B., Mulholland, M. R., & Mopper, K. (2013). Chromatographic determination of nanomolar cyanate concentrations in estuarine and sea waters by precolumn fluorescence derivatization. Analytical chemistry, 85(14), 6661-6666.

l. 495: Did you check the stability of cyanate during your rather long analysis procedure?

The first step of the procedure is to convert cyanate into 2,4(1H,3H)-quinazolinedione, which is a stable compound. Furthermore, we processed cyanate standards with the samples from the very beginning, accounting for any potential loss.

l. 549: "nmol L-1".

Corrected.

l. 596: Mention how you tested the prerequisites for t-tests and ANOVA (i.e., homoscedasticity and normal distribution of residuals).

We amended the text as follows (L595): “Levene’s Test was used to test equality of variances and QQ plot and Kolmogorov Smirnov Test were used to assess normal distribution of residuals. For each extractant, statistical significance of the difference between added and recovered cyanate was tested using t-test on raw data, where F-test was used for testing equality of variances.”

l. 781: Replace 0.01 M by 10 nM CaCl2 for consistency with the text.

We use throughout the manuscript consistently “0.01M”.

l. 768: Do not include “redrawn from” in the superscript. And shouldn’t it be “redrawn from ref. 49” or “from name of author(s)” and 49 as superscript?

Thank you. Fixed.

Fig. 4: There are two subfigures “D”. Please explain the meaning of the lines in subfigures B-D.

Thanks. We changed the last panel to “E” and added the following text to the legend to explain the lines in b-d: “Urea concentration over time was described by a first order reaction (eq. 15), and ammonium and cyanate concentrations were fitted with a third and fourth degree polynomial function, respectively (eq. 16 and 17).”.

Reviewer #2 (Remarks to the Author):

The manuscript (Cyanate – low abundant but actively cycled nitrogen compound in soil) by Mooshammer et al. attempts to describe cyanate cycling in terrestrial systems. Overall, I found the description and experiments very compelling and am intrigued by the role that this process may play in many different ecosystems. The relative paucity of other reports on this phenomenon in terrestrial systems combined with the potential impacts this could have on broader biogeochemical cycles makes this a study that will likely be interesting to a wide range of readers. I can point out only a few (relatively minor) points for potential clarification as listed below:

Thank you very much for your positive feedback.

• If I understand correctly, the isotope tracer experiments are used to estimate the cyanate turnover rates in soil. Lines 455-457 indicate that the cyanate added to soil was approximately 250 times the typical concentrations. Given this, how does the reported biotic half life of cyanate (generated from tracking the decay of a relatively large burst of added material) reflect turnover at more natural concentrations?

As mentioned in the Materials and Method section, in a preliminary experiment with cyanate added at natural concentrations, we found that the depletion was very rapid and we were unable to accurately quantify it. To verify that the results on the turnover of cyanate at high concentrations was not biased, we compared them to the results of the second experiment (“Urea addition experiment”), where cyanate concentrations were in the range of natural concentrations. In the original manuscript, we discussed this in lines L267-304 and the data

are presented in Table 1. Both experiments show similar results on cyanate turnover, lending support to the experiment with the high cyanate addition. Furthermore, we show that the turnover of cyanate derived from the high cyanate addition is similar to a urea fertilization event in an agricultural soil ("Urea addition experiment") but higher than under unamended conditions.

To make this clear earlier in the text we added the following: "Preliminary experiments indicated rapid consumption of added cyanate. Thus, to avoid fast depletion of the added cyanate pool, we added approximately 250-fold the *in-situ* cyanate concentration. For comparison of cyanate turnover between the two experiments see below discussion on mean residence time and gross cyanate production.

• The isotope tracer experiment was very interesting and I appreciate the mention/description of the method used for this. In addition to tracking the loss of cyanate, was this approach able to identify any other fates of the 15N-labeled cyanate? Or, could other approaches be applied in the future to determine whether any of this nitrogen was entering into biomass pools (microbial, plant or otherwise)? The discussion in lines 254-262 highlight potential uptake into microbial biomass but any tracking of the 15N tracer into the biomass pool would be extremely compelling if this were available.

It would indeed be very interesting to better understand the fate of consumed cyanate. However, tracing ^{15}N from cyanate into other pools, including microbial biomass, ammonium or nitrate, is challenging. Given the high nitrogen background in those pools it is difficult to detect the ^{15}N from cyanate, unless a large amount of ^{15}N-labelled cyanate is added. Even adding 250 times the *in-situ* concentration, as done in the isotope tracer experiment, would have not contained enough ^{15}N that could be traced into other pools. NanoSIMS single-cell analysis may be more sensitive, but nevertheless even this would require larger amounts of the tracer to be added to the soil.

o Similarly, were water extracts or other fractions from the samples that may contain microbial metabolites considered for analysis via an Orbitrap method for identification of 15N containing compounds?

Same as above – this would be very interesting but is unfortunately extremely challenging in terms of required concentration. An Orbitrap mass spectrometer (MS) would not be sensitive enough to detect differences in the ratio of $^{15}N/^{14}N$. An isotope ratio mass spectrometer (IRMS) would offer a higher sensitivity.

• Lines 380-381: There is mention of samples being stored at -80 deg C as there is less decay of material over time. Can the authors comment on typical storage times between sample collection and analysis?

The study by Widner et al. (2013) showed that cyanate was stable at -80°C over a period of 270 days. We now mention this in the revised text (L384). All experimental work for this manuscript was done within 2 years, where the storage time of samples ranged between few days to a maximum of few months.

• Figure 5: The authors identified a marked distinction between NH4+/NCO- typical of terrestrial versus marine settings. Can the authors speculate on why this may occur? Is this driven by the low turnover of cyanate the authors mention (lines 337-340) and, if so, any potential reasons for the low turnover rate in marine settings versus terrestrial?

Great point. The observed differences in the relative concentration and turnover of cyanate between terrestrial and marine ecosystems could be due to several factors, including differential stability, abiotic and biotic sources as well as degradation pathways, and other abiotic and biotic interactions or factors unknown today. Exploring the potential main drivers of cyanate dynamics between different ecosystem types requires a better understanding of not only the stability of cyanate but also the partitioning of sources and fate of cyanate. Given our limited understanding of cyanate dynamics in environment at this point, we would prefer not to speculate in the manuscript about this point.

• A couple potential typos to consider:
o Line 131: presumable the authors intend "…/ammonium and carbon dioxide/…" (instead of "and on dioxide"

Corrected. Thank you.

o Figure 4: Has two panels labeled "D" and no panel "E" in the figure (but E is listed in the caption)

Changed the last panel to "E".

Reviewer #3 (Remarks to the Author):

Authors of this work investigate cyanate availability and dynamics in soils, providing evidence that cyanate is actively (and mainly) cycled by biotic processes. The work is original and makes a significant contribution to the understanding of the environmental role and significance of cyanate in terrestrial ecosystems, a matter hitherto almost entirely unknown. The experimental work was nicely designed and conducted, and the results are robust and of general interest. Therefore, I feel that there is merit to publishing the paper without serious modifications.

Thank you very much for your positive feedback.

Comments:

1. Authors found that consumption of isotopically labelled cyanate was substantially higher in grassland soil than in arable soil, and that this consumption was mainly biotic, with only small contributions from abiotic processes. This result should be discussed more deeply, giving a possible explanation for this. Grassland soils are usually nutrient-rich, with fertile upper soil layers, but urea fertilizers are used in arable lands probably resulting in a significant formation of cyanate.

Thank you for this great suggestion. We amended the text as follows: "The difference in cyanate turnover between the two soils could be due to differences in the composition and abundance of organisms expressing cyanases. The grassland soil had a higher soil organic carbon content (37 mg g^{-1}) compared to the arable soil (10 mg g^{-1}). Thus, it is likely that the grassland soil contained a larger microbial community, as microbial biomass scales with soil organic carbon (Hartman & Richardson, 2013). A higher microbial biomass may correlate with a higher abundance of cyanate-degrading organisms.

Hartman, W. H., & Richardson, C. J. (2013). Differential nutrient limitation of soil microbial biomass and metabolic quotients (q CO 2): is there a biological stoichiometry of soil microbes?. PloS one, 8(3), e57127.

2. Although microbial biodiversity is not a goal of this work, a metagenomic analysis to stablish the relative abundance and diversity of the microorganisms able to degrade cyanate in the grassland and arable soils could be an interesting complement to this work.

A metagenomic analysis focusing on the genes encoding cyanases would be indeed interesting. However, the focus of this work was on the biogeochemical aspects for which we developed a new analytical method. The development of this new method was time and resource intensive. Therefore, we feel that performing in addition comparative metagenomic analyses are beyond the scope of this work.

3. Some data and calculations given in Table 1 and discussed in pages 11-12 are estimations that should be taken with cautions. I recommend considering and discussing these data more prudently.

We agree that those estimates should be taken with caution. We state that the presented gross rates are not modelled rates using an isotope pool dilution approach. Using different MRTs we demonstrated that those rate estimates were all in the same order of magnitude. The rates were three orders of magnitude lower than those of N mineralization and nitrification, which is a substantial difference. The goal was to get an estimate of the magnitude of the process rate and compare it to other N cycling process rates. To state this more clearly, we amended the text in L266: "We therefore explored *in-situ* gross cyanate production rates by an alternative approach, **which may not accurately reflect actual rates but still yields first insights into the magnitude of the cyanate flux in soils."**

4. In the titles of the cited references, the scientific names of the organisms should be written in italics.

Done.