

## ***Interactive comment on “Seasonal variations in nitrate isotope composition of three rivers draining into the North Sea” by A. Deek et al.***

**A. Deek et al.**

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We thank the anonymous reviewer who made a number of helpful suggestions. Her/his main concern is that other inorganic and organic forms of N besides nitrate contribute substantially to total N load. While we apologise that pertinent data have not been raised (e.g.,  $\delta^{15}\text{N}$  of ammonia) in the course of our study, we stress that nitrate is always and by a large margin the dominant DIN form in the studied rivers (details below). Thus, a major concern voiced by the reviewer may indeed not apply. While we considered all of the points raised and tried to reconcile our opinions with those of the reviewer wherever it was appropriate, we did not agree with some of the proposed changes or did not believe that criticism offered was justified. Each of these cases is addressed below. In the following, we comment on each point raised by the reviewer (listed with

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numbers) and indicate what changes have been made in our revised manuscript.

1) In particular, measuring  $[\text{NH}_4]$  and  $\text{NH}_4$   $\delta^{15}\text{N}$  would help support their claims and rule out alternative explanations for their observations. A more complete study would at least identify what the dominant N species in the river are – how high is  $[\text{NH}_4]$  relative to  $[\text{NO}_3]$ , and does it vary throughout the year?

In the discussion paper, we presented results from  $\text{NO}_3^-$  concentrations and its  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values, and assumed that  $\text{NO}_3^-$  is the dominant N species without referring to actual measurements or available data sets from authorities monitoring nutrient concentrations in the rivers. Indeed, we did measure  $\text{NH}_4^+$  and  $\text{NO}_2^-$  concentrations for some of the water samples, and thus add a supplemental table listing these data. Unfortunately, we did not measure  $\delta^{15}\text{N}\text{-NH}_4^+$ , but the relevance of ammonia in river water is limited due to the low percentage relative to total DIN (dissolved inorganic nitrogen): From the supplemental table, it is seen that  $\text{NO}_3^-$  is the dominant N species compared to  $\text{NO}_2^-$  (< 2% of DIN) and  $\text{NH}_4^+$  (< 6% of DIN).

Considering the river Weser and Ems, we observed highest ammonium concentrations in winter (October–March), but the seasonal pattern is less pronounced than the seasonality of nitrate concentrations. Considering the Rhine River, we did not measure  $\text{NH}_4^+$  during winter months, but refer to measurements from the regional authority (LfNUV NRW: Landesamt für Natur, Umwelt und Verbraucherschutz NRW: [http://luadb.lids.nrw.de/LUA/gues/probe.php?messstellen\\_nr=000504&ersterAufruf=aktuelle+Werte&yAchse=Standard&hoehe=400&breite=724](http://luadb.lids.nrw.de/LUA/gues/probe.php?messstellen_nr=000504&ersterAufruf=aktuelle+Werte&yAchse=Standard&hoehe=400&breite=724)) that monitors nutrients continuously at the study site in Bimmen-Lobith. According to LfNUV NRW, highest ammonium concentrations (between 4 and 8  $\mu\text{mol/L}$ ) were recorded for January and February 2008. This suggests that ammonium concentrations are slightly higher in winter than in summer months.

2) On p. 6071, the authors claim that  $[\text{NH}_4]$  are high during winter – this would seem

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to support high rates of nitrification being the source of increased [NO<sub>3</sub>] during winter, not decreased rates of NO<sub>3</sub> assimilation, as is suggested on p. 6067-6068.

The expression on p.6071 may be unclear, and we corrected this sentence. Because ammonium comprises less than 6% of total DIN, nitrification of ammonium would not dramatically increase actual nitrate concentrations, in contrast to the interruption of nitrate consumption in winter. Thus, we state that assimilation is the main process determining nitrate concentrations.

3) What about [DON]? ...Similarly, what is the [PN] in these samples? Is it quantitatively relevant. . .?

We did not measure DON concentrations within this study, and data on PN concentrations are not available for these samples. According to a recent study by Schlarbaum et al. (Biogeosciences Discuss., 7, 7543–7574, 2010) in the Elbe, around 23% of the total dissolved nitrogen pool was in the form of reduced dissolved N (RDN). The study of Schlarbaum et al. also established that combined PN and RDN concentrations were, to a first approximation, inversely correlated to nitrate concentrations. Thus, RDN has to be considered when setting up a complete riverine N budget. But the aim of this study was to characterise nitrate and tentatively identify nitrate sources by its isotopic variations. For completeness, we added the weight percentage of C and N of the suspended matter (SM) (supplemental table).

4) Additionally, the authors contradict themselves throughout the text (i.e., characterization of the δ<sup>15</sup>N of NO<sub>3</sub> from agricultural fertilizer as between 4 to 9‰ on page 6054, but then quote it as having a δ<sup>15</sup>N from -5 to 8‰ per mil on p. 6061).

On p. 6054 δ<sup>15</sup>N-NO<sub>3</sub>- we quote δ-values of organic fertilizers, and on p. 6061, δ<sup>15</sup>N-NO<sub>3</sub>- values of organic and synthetic fertilizers. For better understanding, we rephrased some sentences throughout the text.

5) The authors claim nitrification of soil ammonia is the dominant source of NO<sub>3</sub> in

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river water; have the authors confirmed that their results are consistent with the work of Casciotti, 2009; Buchwald and Casciotti, 2010, Casciotti et al., 2010?

Principal statement of our study is that nitrification of soil ammonia is the dominant source of nitrate in river water. Our findings are consistent with several previous studies (i.e., Ahad et al. 2006; Deutsch et al. 2006; Voss et al. 2006) who determined nitrate sources of anthropogenically influenced rivers comparable to the rivers studied here. Recent studies have highlighted uncertainties about the exact source of oxygen in nitrate deriving from nitrification. We are aware of recent findings (p.6063) and mention restraints of the Eq (2) which may need to be revised. We are unsure if it is permissible to extrapolate results from pure culture studies with marine bacteria (Casciotti 2009; Buchwald and Casciotti 2010: *Nitrococcus mobilis*) to our case and data sets, which considers nitrification in soils. We also refer to our response to reviewer 1 on this matter.

6) Did the authors measure changes in [NH<sub>4</sub>] and δ<sup>15</sup>N to confirm this supposition?

Soil ammonium and respective δ<sup>15</sup>N-NH<sub>4</sub><sup>+</sup> were not measured.

7) Additionally, the authors claim that in nitrification, 2 oxygen atoms come from water, and 1 comes from dissolved O<sub>2</sub> gas; this is an old explanation, and newer evidence has shown that this is not the case; see work of Casciotti.

See point 5 above and response to reviewer 1

8) It's not clear to me that just because [NO<sub>3</sub>] and NO<sub>3</sub> δ<sup>15</sup>N are inversely correlated that anthropogenic inputs are the cause of this variation. . .This anticorrelation alone does not mean that NO<sub>3</sub> in the river is dominated by anthropogenic sources.

It may be a misunderstanding, because we did not intend to state that the anti correlation of nitrate and δ<sup>15</sup>N-NO<sub>3</sub>- is caused by the anthropogenic influence (but then we did not find a statement to this effect in our text). We attribute the generally high δ<sup>15</sup>N-NO<sub>3</sub>- to the anthropogenic influence.

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9) p. 6054, lines 10-12: I disagree with this statement; . . . Only sources of N (like precipitation or N<sub>2</sub> fixation) have a characterizable d<sup>15</sup>N; nitrification is a transformation of N from one form (NH<sub>4</sub>) to another (NO<sub>3</sub>).

We agree; it is not correct to mix up sources of N that have isotopic fingerprints with N transformation processes. The sentence has been corrected.

10) p. 6054, lines 16-18: similarly, is the reported range in d<sup>15</sup>N of fertilizer, 4 to 9‰ that of NO<sub>3</sub> in streams affected by fertilizer, in which case denitrification may have elevated the d<sup>15</sup>N of the NO<sub>3</sub>, or is it the d<sup>15</sup>N of directly measured fertilizer?

See 4), in the introduction, δ<sup>15</sup>N-NO<sub>3</sub><sup>-</sup> values of different sources of stream water nitrate are presented.

11) p. 6054, lines 21-23: it would be more appropriate to provide the formal definition

This has been corrected

12) p. 6058, line 22: rephrase to “summer maxima were observed each year”

This has been corrected

13) p. 6058, lines 23-25: are these seasonal differences statistically significant?

A Student's t-test confirmed that summer and winter values in the rivers Weser and Ems are significantly different from each other in their δ<sup>15</sup>N and δ<sup>18</sup>O-NO<sub>3</sub><sup>-</sup> values (p<0.05). Summer and winter δ<sup>15</sup>N and δ<sup>18</sup>O-NO<sub>3</sub><sup>-</sup> values of the Rhine River indicated no significant difference (p>0.1). Thus, we rephrased the description of the seasonal patterns in the results section.

14) p. 6059-6060, lines 25-1: There is not enough evidence to support the claim that the inverse relationship between [NO<sub>3</sub>] and NO<sub>3</sub> d<sup>15</sup>N is due to in-river NO<sub>3</sub> assimilation; this could also be caused by in-river denitrification, or the addition of NO<sub>3</sub> with a low d<sup>15</sup>N during times of high N load. The authors need to constrain the problem more before they can make this claim.

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This remark is a bit confusing (page 6059-6060?), because the inverse relationship between NO<sub>3</sub><sup>-</sup> and δ<sup>15</sup>N-NO<sub>3</sub><sup>-</sup> is discussed in detail in the section “Biological processes in the rivers” (page 6067-6069). Here, we state that water column denitrification is unlikely to occur in the well oxygenated water column (p. 6067, l 24-26). Also, we state that drainage and ground water may permanently add diffuse nitrate inputs (p.6068, l. 16-24). Indeed, we expected that high discharge events may add NO<sub>3</sub><sup>-</sup> with low δ<sup>18</sup>O values; as observed in a previous study in the Elbe River (Johannsen et al., 2008), but during this study, we did not observe single flood events accompanied by an immediate response of δ<sup>18</sup>O or δ<sup>15</sup>N.

15) p. 6060, top paragraph; a correlation between PN d<sup>15</sup>N and degree of anthropogenic impact does not mechanistically explain the absolute value of PN d<sup>15</sup>N; this would require knowledge of the d<sup>15</sup>N of the sources of N to the rivers (i.e., direct measurement of the d<sup>15</sup>N of the fertilizer being applied, the d<sup>15</sup>N of the NO<sub>3</sub> in rain, etc.), as well as rate measurements of the processes (denitrification, NO<sub>3</sub> assimilation, etc.), to explain the absolute value of PN d<sup>15</sup>N.

We state that the relatively high δ<sup>15</sup>N-PN value observed in this study is comparable to previously observed δ<sup>15</sup>N-PN values in non-pristine rivers. Of course, an extended study including measurements of rates and isotopic signatures would be advantageous. However, the δ<sup>15</sup>N-PN values presented in this study are the first data raised for these rivers and should be considered as a range of δ<sup>15</sup>N-PN occurring in this region. We shortened this section and deleted some speculative statements.

16) p. 6060, lines 16-20: denitrification in the rivers could also raise the NO<sub>3</sub> d<sup>15</sup>N to the observed values

In the latter section on p. 6067, l 24-26, we state that water column denitrification is unlikely to occur in the well oxygenated water column.

17) it is not clear that NO<sub>3</sub> d<sup>15</sup>N is high because of anthropogenic impact

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The section "Nitrate from organic and synthetic fertilizers" has been rephrased for better understanding.

18) p. 6060-6061: "All these sources are known to contribute to nitrate stocks in surface and groundwater, and each has more or less specific isotopic compositions"; I disagree with this assumption; this is an oversimplified interpretation of the cited reference.

This section has been rephrased.

19) p. 6061, this is a confusing summary of the  $\delta^{15}\text{N}$  of fertilizer, and more over, it is not relevant to the interpretation of the data; as the authors describe elsewhere, the initial  $\delta^{15}\text{N}$  of fertilizer will not necessarily be reflected by the  $\delta^{15}\text{N}$  of  $\text{NO}_3$  (or  $\text{PN}$ ,  $\text{NH}_4$ , etc) measured in a river, because of 1) the potential for incomplete conversion of substrate (fertilizer) to product ( $\text{NO}_3$ , in this case), 2) mixing with other sources, such as atmospheric deposition, groundwater, etc., with different isotopic compositions, and 3) removal of  $\text{NO}_3$  by fractionating processes such as denitrification and/or assimilation.

This section has been rephrased, but for better understanding the relationship between high  $\delta^{15}\text{N}$ - $\text{NO}_3^-$  and anthropogenic influence (as suggested in 17), we have to introduce the characteristic  $\delta^{15}\text{N}$ - $\text{NO}_3^-$  values.

20) None of these other sources or processes are quantified or constrained in this manuscript, making it difficult to interpret the data with any degree of certainty. Moreover, the lack of a mass and isotopic balance (i.e., the authors do not measure the concentration of other dissolved N species in the river, so we do not know what the dominant form of N is; in particular  $[\text{NH}_4]$  would have been very useful in this study) makes it impossible to interpret the data.

Refer to 1)

21) Indeed, even though the authors measured  $[\text{PN}]$ , they do not report it . . . data that is critical to this explanation, even though they presumably have the data.

For completeness, we added a supplemental table (Tab. 3) with the weight percentage  
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of C and N, but, we do not focus on mass or isotopic budget.  $\delta^{15}\text{N}$ - $\text{PN}$  values should be considered as a range occurring in these rivers. We agree that the section on p. 6069-6070 is rather speculative, thus we have shortened this section and mentioned the limitations of our approach.

22) p. 6063-6064: the discussion of nitrifying bacteria, especially the discussion of groups of nitrifying bacteria, seems unnecessary. . .

The discussion about nitrifiers has been cut.

23) p. 6066, lines 17-25: the  $[\text{NO}_3]$  (and  $[\text{NH}_4]$ ) in precipitation can be quite high in anthropogenically affected areas like the ones in this study; additionally, the  $\delta^{15}\text{N}$  of this  $\text{NO}_3$  and  $\text{NH}_4$  in rain is low (Hastings et al., 2003; Knapp et al., 2010); however, this source of N to the rivers is not addressed in this text (even though the  $\delta^{18}\text{O}$  of  $\text{NO}_3$  in rain is) . . . alternatively, they should drop the whole section – as it is inconsistent.

In a study by Deutsch et al. (2006) who investigated nitrate sources of a small river in Eastern Germany, nitrate concentrations in precipitation varied between 20 and 140  $\mu\text{mol/L}$ . Although these are relatively high concentrations, the direct contribution to the riverine nitrate pool was negligible, because the characteristically high  $\delta^{18}\text{O}$ - $\text{NO}_3^-$  values (Durka et al. 1994) of rainwater nitrate are not noticed in riverine nitrate. This is consistent with findings by Deutsch et al. (2006) and Burns and Kendall (2002).  $\delta^{18}\text{O}$ - $\text{NO}_3^-$  value are more useful to estimate the relevance of atmospheric deposition, in contrast to  $\delta^{15}\text{N}$ - $\text{NO}_3^-$  which may overlap with other sources. Thus, we do not discuss  $\delta^{15}\text{N}$ - $\text{NO}_3^-$  values in this section. To sum up, the section "Atmospheric deposition" discusses a source which is not very relevant and thus has been cut

24) p.6067, lines 14-17: "Because assimilation is low in winter (as indicated by the indirect, but significant, negative correlation between  $\text{NO}_3$  concentration and water temperature; Table 2), the more intense soil source and low consumption rates result in higher nitrate loads in winter." I argue that this is a bad assumption. . .

This assumption has been also questioned in 14), and we argued that significant denitrification in the water column can be excluded. Also, nitrification in the river water is unlikely to change the isotopic value of nitrate significantly due to the relatively low proportion of ammonium compared to the nitrate pool (1) +2)). However the introduction of a different water mass with different  $\delta^{15}\text{N-NO}_3^-$  value may influence the isotopic value but will be overlapped by the isotopic effects associated with the interruption of biological activity in winter months.

25) p. 6068: the authors assume that sedimentary denitrification has no isotope effect, and so it should not play a role in producing  $\text{NO}_3^-$  with a high  $\delta^{15}\text{N}$  in these samples. That would be true if, as is the case in ocean sediments, rates of denitrification were diffusion-limited; however, given the high loading of  $\text{NO}_3^-$  in soil pore waters in agricultural soils, this may not be a good assumption. Indeed, the isotope effect for denitrification may be expressed to some degree in soil waters, which would impact the  $\delta^{15}\text{N}$  of  $\text{NO}_3^-$  in these samples.

This remark seems to be a misunderstanding, because we refer to studies on denitrification in sediments (Sebilo et al. 2003; Reinhardt et al. 2006; Lehmann et al. 2007), and extrapolated these findings to the riverine sediments. Within this section (“Biological processes in the rivers”), we do not discuss denitrification in soils (this point was shortly mentioned in the section “Nitrate from organic and synthetic fertilizers”). Because both sections lead obviously to misunderstandings, we rephrased the respective sections (similar to 14) +17))

26) ...I assume that the authors are claiming that eukaryotic biological activity decreases in the winter (although they provide no direct evidence of this); however, they show no evidence that prokaryotic activity decreases in the winter.

Because we do not provide measurements to determine prokaryotic or eukaryotic activity, we prefer the more general expression of biological activity.

27) The figures are hard to read – please make them bigger, especially Figs 3, 5

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→Remark to the editorial support to change the format.

28) Fig 6 – use  $\ln(f)$  on the x axis instead of  $f$  to get a linear relationship to estimate fractionation factor.

We could change the x-Axis as illustrated in Fig. 6b. But we remark that a plot of  $\delta^{15}\text{N-NO}_3^-$  and  $\delta^{15}\text{O-NO}_3^-$  versus the fraction of remaining reactant nitrate pool better illustrates the consumption efficiency as discussed on page 6069.

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Date	River	Watercolumn			Suspended Matter		
		NO <sub>3</sub> [μmol/L]	NO <sub>2</sub> [μmol/L]	NH <sub>4</sub> <sup>+</sup> [μmol/L]	N [%]	C [%]	C <sub>org</sub> [%]
20.03.2008	Rhine	229.4	1.5	3.2	0.4	4.6	3.9
03.04.2008		207.6	1.3	3.0	0.5	5.5	4.2
17.04.2008		191.1	1.4	3.1	0.4	5.4	4.3
15.05.2008		131.3	0.3	1.8	0.7	6.9	4.8
29.05.2008		140.2	0.4	1.9	0.7	8.2	4.9
12.06.2008		147.8	0.4	2.2	0.5	5.7	4.9
26.06.2008		129.9	0.3	2.2	0.9	9.7	7.7
04.12.2007	Weser	285.2	1.5	6.5	0.5	6.6	5.8
18.12.2007		283.7	1.4	6.0	0.3	4.3	3.3
31.12.2007		286.5	1.7	9.4	0.3	3.8	3.2
15.01.2008		317.6	2.2	8.9	0.3	4.7	4.1
29.01.2008		291	1.8	7.1	0.3	3.4	3.2
12.02.2008		292.3	1.6	6.3	0.4	5.2	4.1
26.02.2008		290.8	1.5	7.8	0.4	5.7	5.6
11.03.2008		303.9	1.4	8.1	bdl	7.3	6.1
25.03.2008		295.5	1.5	7.3	0.5	5.4	4.4
08.04.2008		210.7	0.9	5.8	0.4	4.8	4.1
22.04.2008		260.9	0.7	2.8	0.6	5.7	5.1
06.05.2008		208.2	0.7	4.4	0.9	6.7	6.4
03.06.2008		161.5	1.4	8.6	1.3	9.1	7.9
07.06.2008		184.8	1.7	6.3	1.6	11.4	9.0
01.07.2008		173.4	1.4	7.7	0.7	7.3	7.7
02.12.2008		241.1	1.2	7.4	0.7	7.2	6.4
27.01.2009		268.7	2.2	17	0.9	9.2	9.3
24.02.2009		312.7	2.2	9.2	0.8	7.6	8.1
24.03.2009		218.3	1.1	3.4	0.6	6.8	6.7
21.04.2009		174.8	0.6	1.6	1.6	12.0	12.5
19.05.2009		117.1	0.8	2.3	1.3	10.3	10.7
16.06.2009		147.2	1.5	4.4	1.5	10.2	10.6
16.07.2009		123.7	1.4	5.7	1.7	13.5	12.7
11.08.2009		136.2	1.1	7.8	0.9	8.3	7.7
08.09.2009		156.4	0.6	3.7	1.2	11.7	9.3
23.11.2009		281.4	1.4	4.0	0.7	6.9	8.3
20.12.2007	Ems	447.8	3.1	18.2	0.8	10.9	9.7
18.02.2008		391.2	2.9	14.5	1.2	14.6	10.8
28.02.2008		311.2	3.2	13.5	1.3	14.1	13.4
13.03.2008		335.7	2.3	11.7	1.5	13.6	13.0
14.04.2008		338.4	2.4	8.7	2.2	16.8	12.5
18.04.2008		325.6	3.3	11.4	3.9	28.4	25.4
24.04.2008		285.9	1.7	1.7	3.5	20.4	17.2
06.05.2008		230.2	2.4	2.0	4.8	25.6	22.3
23.05.2008		289.8	6.0	10.2	3.8	24.4	22.2
04.06.2008		223.5	4.6	10.6	4.5	29.8	26.9
20.06.2008		232.1	1.6	2.6	2.0	15.5	14.3

Fig. 1. Supplemental table: DIN concentrations and weight percentage of SM.

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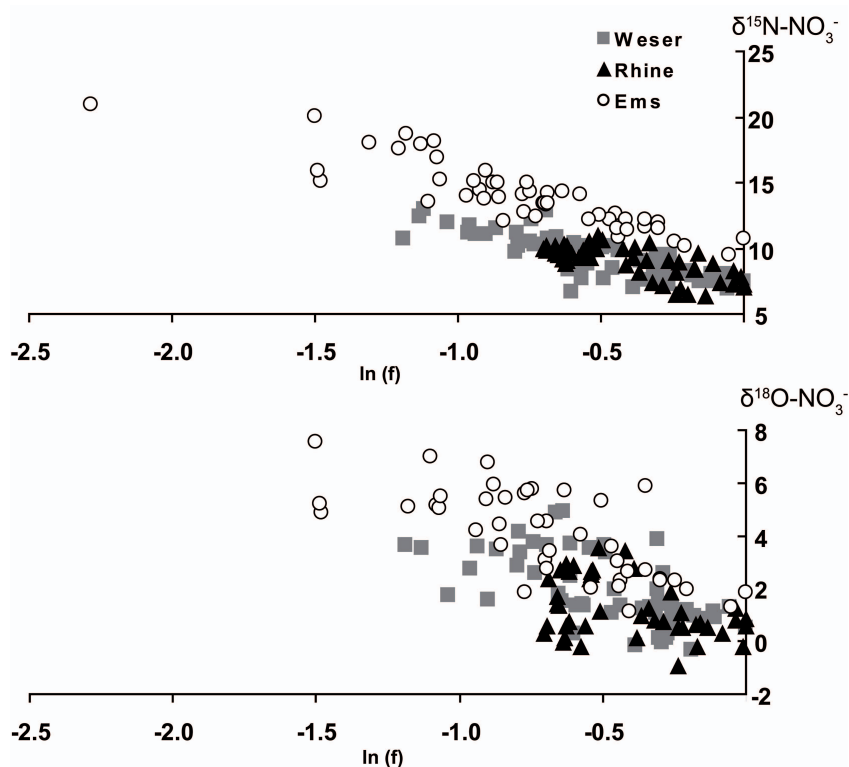


Fig. 2. Fig. 6b:  $\delta^{15}\text{N-NO}_3^-$  and  $\delta^{18}\text{O-NO}_3^-$  versus  $\ln(f)$

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