\[^{15}\text{N} \text{ gas-flux method to determine } \text{N}_2 \text{ emission and } \text{N}_2\text{O pathways: a comparison of different tracer addition approaches}\]

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Abstract. \textsuperscript{15}\text{N} gas flux method allows for quantification of \text{N}_2 flux and tracing soil N transformations. An important requirement for this method is a homogeneous distribution of the \textsuperscript{15}\text{N} tracer added to soil. This is usually achieved by soil homogenization and admixture of the \textsuperscript{15}\text{N} tracer solution or multipoint injection of tracer solution to intact soil. Both methods may create artefacts. We aimed at comparing the results of the gas flux method using both tracer distribution approaches.

Intact soil cores with injected \textsuperscript{15}\text{N} tracer solution show wider range of the results obtained. Homogenized soil shows better agreement between repetitions, but significant differences in \textsuperscript{15}\text{N} enrichment measured in soil nitrate and in emitted gases were also observed. For intact soil the wider variability of measured values rather results from natural diversity of non-homogenized soil cores than from inhomogeneous label distribution. Generally, comparison of the results of intact cores and homogenized soil did not reveal statistically significant differences in \text{N}_2 flux determination. In both cases, pronounced dominance of \text{N}_2 flux over \text{N}_2\text{O} flux was noted. It can be concluded that both methods showed close agreement and homogenized soil is not necessarily characterized by more homogenous \textsuperscript{15}\text{N} label distribution.
1. Introduction

Determination of soil nitrogen transformation pathways and quantification of gaseous N emissions often requires soil incubation experiments including significant manipulations of natural soil conditions. Especially, the quantification of soil N\(_2\) flux in field studies is very challenging due to high atmospheric background. The most common method for both detailed tracing of soil N transformations and determination of N\(_2\) emission is the application of \(^{15}\)N tracer (Aulakh et al., 1991; Baily et al., 2012; Bergsma et al., 2001; Buchen et al., 2016; Deppe et al., 2017; Kulkarni et al., 2013; Morse and Bernhardt, 2013; Müller et al., 2014; Müller et al., 2004; Well et al., 2019). However, this implies a significant impact for the soil due to additional fertilization and soil disturbance depending on the way of tracer addition. For the tracer addition several different strategies may be applied. The two most common techniques are: soil homogenization where the tracer solution is mixed with the soil, or usage of intact soil cores where tracer solution is added through multiple needle injections. Both methods lead to potential bias. Following soil homogenization, the soil structure is changed through sieving and mixing, roots and stones are removed, but this results in the best achievable homogeneity of soil properties and tracer distribution within the soil column and thus better comparability between the repetitions. For needle injections, the soil structure stays unchanged but the pointwise injection may not ensure the homogenous distribution of the tracer which is crucial for the proper application of \(^{15}\)N gas flux method. Moreover, incomplete equilibration of water content after injecting aqueous tracer solution could lead to increased wetness near the injection spots and thus to enhanced denitrification.

Here we aimed at comparing the results of these different strategies and test how far the determined \(^{15}\)N pool derived N\(_2\) and N\(_2\)O fluxes are altered due to a particular soil treatment.

2. Methods

2.1 Experimental set-up

Three treatments were applied: (1) soil was sieved with 4mm mesh size, the tracer solution was added evenly, soil was homogenized and packed into the incubation column (treatment H+M: homogenized + mixed); (2) intact soil cores were directly collected into the incubation columns and the tracer solution was added through the injection needles in 12 homogeneously distributed injection points in 6 depths (in total 72 injection points per column) (treatment I+I: intact + injected); (3) soil was sieved with 4mm mesh size (like in treatment H+M), packed into the incubation column, and the tracer solution was added through the injection needles (like in treatment I+I) (treatment H+I: homogenized + injected). For each treatment the soil columns were 0.3 m high with diameter of 0.15 m. Silt loam soil Albic Luvisol from arable cropland of Merklingen experimental station (Germany) was used (silt content approx. 87%, 11% clay, 2% sand). The soil density of intact cores was 1.3 g ml\(^{-1}\) and the packed columns were compacted to the same density, which gave 6.89 kg soil per column.

For each soil column, 216 mL of 319 mg N L\(^{-1}\) NaNO\(_3\) solution with 73 at% \(^{15}\)N was added. This resulted in the following initial experimental settings: 75% water-filled pores space (WFPS), 37 mg N kg\(^{-1}\) NO\(_3\), 42.5 at% \(^{15}\)N measured in the
subsamples of the homogenized soil. The incubation lasted 8 days. The columns were continuously flushed with a gas mixture with reduced \( \text{N}_2 \) content to increase the measurements sensitivity (2% \( \text{N}_2 \) and 21% \( \text{O}_2 \) in \( \text{He} \), (Lewicka-Szczebak et al., 2017)) with a flow of 10 mL min\(^{-1}\). The gas samples were collected daily in the first 4 days and every second day in the last 4 days into two 12 mL septum-capped Exetainer® (Labco Limited, Ceredigion, UK) connected to the vents of the incubation columns.

2.2 Gas analyses

The gas samples were analysed with a modified GasBench II preparation system coupled to MAT 253 isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany) according to Lewicka-Szczebak et al. (2013). In this set-up, \( \text{N}_2\text{O} \) is converted to \( \text{N}_2 \) prior to analysis, which allows simultaneous measurement of stable isotope ratios \( ^{29}\text{R} \left( ^{28}\text{N}_2/^{29}\text{N}_2 \right) \) and \( ^{30}\text{R} \left( ^{28}\text{N}_2/^{30}\text{N}_2 \right) \), of \( \text{N}_2 \), of the sum of denitrification products (\( \text{N}_2+\text{N}_2\text{O} \)) and of \( \text{N}_2\text{O} \). Based on these measurements the following values are calculated according to respective equations presented previously in (Lewicka-Szczebak et al., 2017) (after Spott et al., 2006):

- The fraction originating from the \( ^{15}\text{N} \)-labelled pool (\( f_p \)) (Eq.1 in (Lewicka-Szczebak et al., 2017)) for \( \text{N}_2 \) (\( f_{p,\text{N}_2} \)), \( \text{N}_2+\text{N}_2\text{O} \) (\( f_{p,\text{N}_2+N_2\text{O}} \)) and \( \text{N}_2\text{O} \) (\( f_{p,\text{N}_2\text{O}} \)) within the sample;
- \( ^{15}\text{N} \) abundance of \( ^{15}\text{N} \)-labelled pool (\( a_p \)) (Eq.3 in (Lewicka-Szczebak et al., 2017)) from which \( \text{N}_2 \) (\( a_{p,\text{N}_2} \)) or \( \text{N}_2\text{O} \) (\( a_{p,\text{N}_2\text{O}} \)) originate;
- \( \text{N}_2\text{O} \) residual fraction (\( r_{\text{N}_2\text{O}} \)) (Eq.6 in (Lewicka-Szczebak et al., 2017)) representing the unreduced \( \text{N}_2\text{O} \) mole fraction of pool-derived gross \( \text{N}_2\text{O} \) production.

2.3 Soil analyses

At the end of incubation soil samples were collected from each column using a Goettinger boring rod with diameter of 18 mm (Nietfeld GmbH, Quakenbrück, Germany). Three cores were taken from each column, separated in top (0 to 15 cm) and bottom (15 to 30 cm) layer. For injected treatments ((H+I) and (M+I)) these sample cores were taken between injection points and additional cores were collected from injection points. All soil samples were homogenised and analysed for water content (by weight loss after 24 h drying in \( 110^\circ\text{C} \)), nitrate concentration (by extraction in \( 2\text{M} \) KCl 1:4) and \( ^{15}\text{N} \) enrichment in nitrate (by bacterial denitrification method (Sigman et al., 2001)).

2.4 Statistics

For testing the statistical significance of the differences between treatments ANOVA and Tukey HSD Post-hoc test were applied using R 3.4.2 (R Core Team, 2013).
3. Results & Discussion

3.1 Gas fluxes and denitrification product ratio

In order to compare the treatments, the time course of the results has to be taken into account because the gas production differed largely between the sampling dates (Fig.1). Therefore, we checked for statistically significant differences between the treatments individually for each sampling date. The results show well comparable trends and no statistically significant differences between treatments (Fig.1). Notably, $r_{N2O}$ shows very good agreement at the beginning of the experiment, when the large gas concentrations were measured, and start to differentiate when the fluxes drop from the 3rd day (Fig. 1D), but these differences are not statistically significant. However, if the experiment is evaluated for the cumulative values, significant differences between treatments appear (Table 1). The cumulated gas fluxes of N$_2$O and N$_2$ are significantly different between the treatments I+I and H+I, whereas the H+M treatment does not differ significantly from the others. However, comparison of the entire denitrification gas flux (joint N$_2$+ N$_2$O flux) reveals no statistically significant difference between treatments (Table 1). Product ratios are compared as cumulated $r_{N2O}$ (calculated with the cumulated fluxes) and mean $r_{N2O}$ (average value of all sampling points). Cumulated $r_{N2O}$ shows identical pattern of significant differences as the cumulated N$_2$ and N$_2$O fluxes. For mean $r_{N2O}$ values, H+M and H+I treatment are significantly different, whereas the I+I treatment does not differ significantly from the others.

There results show that the different tracer application strategies tested had no impact on the total denitrification (N$_2$+ N$_2$O), but the product ratio may be slightly shifted, which results in differences by comparing separately N$_2$ or N$_2$O flux. This presumably results from the differences in distribution of moisture and nitrate between treatments (see Sect. 3.2). Anyway, all determined $r_{N2O}$ values, although partially different, indicate pronounced dominance of N$_2$ over N$_2$O emission.

Importantly, no significant differences were noted between the H+M and I+I treatment, only H+I treatment shows higher N$_2$O flux, lower N$_2$ flux and higher $r_{N2O}$. In this treatment we may deal with joint artefacts associated with soil homogenization and needle injection technique.

The homogenized treatments show better comparability between the repetitions—they show lower standard deviations for gas emissions and for $r_{N2O}$ (Table 1) and also smaller error bars for the daily measurements (Fig.1). The H+I treatment shows the lowest standard deviations for the cumulative gas emission measurements (Table 1). This indicates that the observed heterogeneity for I+I treatment is not due to needle injection procedure but rather due to intact structure of soil cores, which naturally represent the typical soil heterogeneity.

3.2 Soil parameters

A good insight into columns heterogeneity is also provided by the soil analyses performed at the end of experiment (Table 2). Clearly, I+I treatment shows the largest standard deviations between repetitions. Also the most pronounced differences between top and bottom soil layer can be noted for this treatment, but only soil moisture is significantly lower for the bottom layer. Since this is not the case for H+I treatment it indicates the natural heterogeneity of intact cores rather than a result of
label injection procedure. The values from injection points are never significantly different from samples between injection points (within one treatment) which indicates a good distribution of the tracer solution.

Very significant differences between treatments were observed. I+I treatment shows significantly lower nitrate concentration compared to homogenized treatments. This must be due to initial soil nitrate concentration. The soil was stored for two weeks before experiment. Storing of mixed soil or sieving and homogenization procedures probably intensified N mineralization and formation of additional nitrate. Moreover, H+M treatment shows significantly higher $^{15}$N enrichment of NO$_3^-$ ($a^{15}$NO$_3$) than injected treatments. This may be due to injection procedure where the needles might get partially clogged with soil and then addition of tracer solution was lower than planned. The assumption that the injected volume was lower than the target and thus also lower than the addition of tracer solution to H+M treatment, can also be supported by the slightly lower soil moisture and nitrate concentration of the injected treatments.

3.3 $^{15}$N abundance in soil active pools

Despite the pronounced difference in $^{15}$N content between treatments, the results can be still compared because the $^{15}$N abundance of actively denitrifying pool ($a_P$ value) for each sample is individually calculated based on the distribution of N$_2$ and/or N$_2$O isotopologues. We checked how well these calculated $a_P$ values for N$_2$ and N$_2$O correspond with the respective $^{15}$N enrichment measured in soil nitrate ($a_{NO3}$) and between each other (Table 3). This comparison gives additional information about the distribution of the $^{15}$N label. We calculated the cumulative relative difference (Table 3: cum diff, calculated as a sum of differences in $^{15}$N enrichment of different pools for all 24 samples) which represents the overall deviation between the analyzed pools. Very high difference was noted between $a_P$ values of both gases and $a_{NO3}$ in H+M treatment. This is mostly due to the first two sampling days, where $a_P$ values were significantly lower than $a_{NO3}$ (mean difference of ca. 15 at% $^{15}$N, Fig.2), whereas for the next samplings they corresponded very well (mean difference of ca. 1 at% $^{15}$N, Fig.2). This shows that initially the gases were produced in soil microsites depleted in $^{15}$N compared to the mean soil value. This is the case for all three treatments, however the largest difference is observed for H+M treatment due to highest $a_{NO3}$ values. The absolute mean difference (Table 3: mean abs diff, calculated as a mean of $\text{modulus of differences}$ in $^{15}$N enrichment of different pools) represents the average variation range of the compared values. Here it is clear that for comparison between $a_{P,N2}$ and $a_{P,N2O}$ we obtained quite a good agreement, much better than for comparisons with $a_{NO3}$ (Table 3). This shows that both gases originate mostly from the same soil pool. Importantly, even in H+M treatment where large difference between $a_{NO3}$ and $a_P$ values was noted, the difference between $a_{P,N2}$ and $a_{P,N2O}$ is very low. The fact that $a_{P,N2O}$ shows much closer agreement with $a_{P,N2}$ than $a_{NO3}$ suggests that, in case of missing data on $a_{P,N2}$, which is often the case due to high N$_2$ detection limit of the gas-flux method, rather the $a_{P,N2O}$ should be used than $a_{NO3}$ or a theoretical value on $^{15}$N abundance, as it has been also proposed in previous studies (Bergsma et al., 2001; Stevens and Laughlin, 2001). Interestingly, for I+I treatment lower differences between $a_{NO3}$ and $a_{P,N2O}$ or $a_{P,N2}$ values were obtained, but larger difference between $a_{P,N2}$ and $a_{P,N2O}$ when compared to homogenized treatments (Table 3, Fig. 2). This shows that the multiple injection technique reduced the formation of isolated soil microsites of distinct $^{15}$N enrichment than the $a_{NO3}$ value measured for total.
soil. However, the slightly higher difference between \( a_P \) values for \( N_2 \) and \( N_2O \) suggests not identical origin for both gases, *i.e.*, probable slight admixture of hybrid \( N_2 \) (Spott et al., 2011) since the \( ^{15}N \) enrichment of \( N_2 \) shows lower values than \( N_2O \). This could explain the higher cumulated \( N_2 \) flux for \( H+I \) treatment (Table 1).

### 3.4 Homogeneity of \( ^{15}N \) tracer distribution and accuracy of results

Surprisingly, the inconsistency in \( ^{15}N \) abundance in total and actively denitrifying nitrate soil pools (Fig. 2) indicates largest inhomogeneity at the beginning of the incubation for the homogenized soil, which is then equilibrated after 2 days of incubation. This resulted most probably from the imperfect mixing of the relatively wet (gravimetric water content of 29.3%) silt loam soil and could be due to delayed equilibration of added \( ^{15}N \) solution into the centre of soil aggregates where denitrification rates are probably highest (Sextone et al., 1985). But, importantly, these first two days are also the ones with the highest gas production and close agreement of results between all three treatments (see Fig. 1). This suggests that practically even non-homogeneous distribution of \( ^{15}N \) label and thus heterogeneity in concentration and \( ^{15}N \) enrichment of nitrate in soil does not lead to severe bias in determining denitrification and its product ratio.

This study allows only for comparison of these different treatments but not for checking with the true emission values, since we have not used any independent method for fluxes determination. However, what can be observed here is the fact that pronounced differences were observed for \( d^{^{15}N} \) values of different treatments and different pools, but the calculated results for gas fluxes and product ratios were mostly not significantly different between the treatments. This supports the assumption that in real soil situation even the imperfect label distribution allows for obtaining accurate results (Arah, 1997; Deppe et al., 2017). But, importantly, this is possible only if we measure and use \( a_P \) values representing the \( ^{15}N \) values of the pools actively producing \( N_2 \) and \( N_2O \). The fluxes would be significantly underestimated if the \( a_{NO3} \) value was applied for calculations, *e.g.*, for the first sampling point this would result in about 20% underestimation of the \( N_2 \) flux when the measured final \( a_{NO3} \) value was applied, and about 30% underestimation when the initial \( a_{NO3} \) value was applied. Significant differences in \( ^{15}N \) enrichment of total and active nitrate pool has been also found in our previous laboratory and field studies (Buchen et al., 2016; Deppe et al., 2017). It was shown that in such cases the \( ^{15}N \) enrichment of \( N \) pool undergoing denitrification is well represented by \( a_P \) values, but not by \( a_{NO3} \) values.

The homogeneity of \( ^{15}N \) label distribution depends not only on the fertilizer application technique but even more on the soil type, water content, initial nitrate and ammonium content. In our previous laboratory experiments quite a good agreement between \( a_{NO3} \) values and \( a_P \) values was achieved indicating a homogenous denitrifying pool (Lewicka-Szczebak et al., 2017). In that study similar soil texture was used (silt loam), but initial amount of nitrate and ammonium was very low, and soil samples were prepared at soil moisture of 70% WFPS and rest water was added on top, and soil was incubated in high moisture conditions. But notably, the anoxic conditions showed perfect agreement in \( a_{NO3} \) and \( a_P \) values whereas for oxic conditions slight differences have also been noted (Lewicka-Szczebak et al., 2017). Oxic conditions can be expected to yield greater disagreement between \( a_{NO3} \) and \( a_P \) due to absence of nitrification in anoxic microsites and thus less dilution of the \( ^{15}N \) label by soil-derived \( N \) sources (Deppe et al., 2017). In the H+M treatment of the actual experiment, inhomogeneity was
probably the result of soil homogenization by too high soil moisture (75% WFPS) due to formation of larger aggregates. But this problem can be overcome if the $^{15}$N label is incorporated at low soil moisture and target moisture is established by adding water afterwards (Lewicka-Szczebak et al., 2017, Well et al., 2019).

Conclusions

Soil homogenisation reduced the variability within soil column and between repetitions but not necessarily improved the $^{15}$N label distribution. Wet homogenisation has lead to uneven label and process distribution. Multiple needle injection of $^{15}$N solution resulted in better agreement between $^{15}$N enrichment of soil and emitted gases, indicating even more homogeneous $^{15}$N label distribution than homogenised treatments.

Larger heterogeneity of intact soil cores, noted as larger deviations of all measured values, reflects the natural soil conditions rather than inhomogeneous $^{15}$N label distribution. Importantly, the results obtained with homogenised soil and with intact soil cores do not differ significantly in the determined $N_2$ flux and denitrification product ratio. Hence, when applying each of these treatments very similar general conclusions will be driven, i.e., the dominance of the $N_2$ flux over the $N_2O$ flux. This is thanks to calculation method applying $a_P$ values determined individually for each sample which assures the adequate results for flux calculation, even by existence of multiple N pools. It was found that $a_{NO_3}$ values can pronouncedly differ from the $a_P$ value of produced gases and its application for $N_2$ flux determination may result in large bias.

Data availability. Original data are available upon request. Material necessary for this study findings is presented in the paper.

Author contribution. DLS and RW designed the experiment and DLS carried it out. Both authors interpreted the results. DLS prepared the manuscript with significant contribution of RW.

Competing interests. The authors declare that they have no conflict of interest.

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Figure 1: Comparison of the temporal changes in N$_2$O concentration (A), fraction of $^{15}$N-pool derived N$_2$O (B), N$_2$ concentration (C), and N$_2$O residual fraction (D) in three treatments: homogenized soil mixed with fertilizer (black points), intact soil cores with fertilizer added through needle injection (red triangles), and homogenized soil with fertilizer added through needle injection (green squares). Error bars represent the standard deviation of 4 replicates within one treatment. Indicated are the statistically significant differences for $p<0.01$ (**) and $p<0.001$ (***)..

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Figure 2: Comparison of $^{15}$N abundance in total initial and final soil nitrate ($a^{15}$N$_{NO_3}$) and in active soil pool emitting N$_2$ ($a_p^{15}$N$_{N_2}$) and N$_2$O ($a_p^{15}$N$_{N_2O}$) in three treatments: homogenized soil and mixed fertilizer (H+M, black points)), intact soil core and injected fertilizer (I+I, red points), homogenized soil and injected fertilizer (H+I, green points).
Table 1: Comparison of cumulated fluxes, cumulated product ratio (cum $r_{N_2O}$) and mean product ratios (mean $r_{N_2O}$) in three treatments: homogenized and mixed (H+M), intact and injected (I+I), homogenized and injected (H+I). Statistically significant differences are indicated (*p<0.05, **p<0.01, ***p<0.001).

| Treatment | cum $N_2O$ [mgN kg soil$^{-1}$ day$^{-1}$] | cum $N_2$ [mgN kg soil$^{-1}$ day$^{-1}$] | cum $N_2$+$N_2O$ [mgN kg soil$^{-1}$ day$^{-1}$] | mean $r_{N_2O}$ | mean $r_{N_2O}$ |
|-----------|--------------------------------------|--------------------------------------|--------------------------------------|----------------|----------------|
| H+M       | 0.63 ± 0.10 ab                       | 2.16 ± 0.31 ab                       | 2.80 ± 0.38 a                        | 0.23 ± 0.05 ab | 0.16 ± 0.14 a  |
| I+I       | 0.55 ± 0.26 a                        | 2.62 ± 1.08 a                        | 3.16 ± 1.18 a                        | 0.18 ± 0.14 a  | 0.25 ± 0.14 ab |
| H+I       | 0.69 ± 0.05 b**                      | 1.83 ± 0.20 b                        | 2.53 ± 0.23 a                        | 0.27 ± 0.04 b**| 0.32 ± 0.15 b***|

Table 2: Soil analyses at the end of the experiment: mixed samples, and separately from the top and bottom layer and for injected columns also from injection points. Statistically significant differences are indicated with uppercase letters (**p<0.01, ***p<0.001).

| Treatment | Sample   | WFPS [%]  | mean NO$_3^-$ conc. [mg N kg$^{-1}$] | mean $a^{15}N_{NO3}$ [at%] | mean $a^{15}N_{NO3}$ [at%] |
|-----------|----------|-----------|-------------------------------------|---------------------------|---------------------------|
| H+M       | Top      | 71.5 ± 0.4 a | 71.8 ± 0.6 a | 35.5 ± 0.5 a | 41.2 ± 0.5 a | 41.3 ± 0.4 a*** |
|           | Bottom   | 72.1 ± 0.8 a |           | 35.2 ± 0.3 a |                      |                      |
| I+I       | Top      | 72.3 ± 2.0 b** |       | 28.6 ± 5.5 a | 29.3 ± 2.7 a |                      |
|           | Bottom   | 65.3 ± 1.8 b** | 68.9 ± 3.5 a | 22.5 ± 2.7 a | 36.1 ± 7.0 a | 32.8 ± 5.9 b |
|           | Injection | 69.0 ± 1.9 ab |           | 26.4 ± 3.6 a |                      |                      |
| H+I       | Top      | 69.7 ± 2.3 a | 69.6 ± 1.9 a | 32.6 ± 0.4 a | 30.9 ± 1.2 a |                      |
|           | Bottom   | 70.2 ± 1.3 a |           | 33.0 ± 0.8 a | 33.7 ± 1.8 a | 31.3 ± 3.0 b |
|           | Injection | 68.8 ± 2.1 a |           | 30.7 ± 1.7 a |                      | 29.2 ± 3.9 a |

Table 3: Differences between the measured $^{15}$N abundance in soil nitrate ($a_{NO3}$) and determined $^{15}$N abundance of $^{15}$N-pool derived N$_2$ ($a_{P,N2}$) and N$_2$O ($a_{P,N2O}$) expressed as the cumulative relative difference for all samples (n=24) (cum diff = $\sum_{i=1}^{n}((a_1 - a_2))/n$). In the above equations $a_1$ and $a_2$ represent the $^{15}$N enrichment of two compared pools ($a_{NO3}$ or $a_{P,N2}$ or $a_{P,N2O}$).

| Difference | $a_{NO3}$-$a_{P,N2}$ | $a_{NO3}$-$a_{P,N2O}$ | $a_{P,N2O}$-$a_{P,N2}$ |
|------------|----------------------|----------------------|----------------------|
|            | cum diff [15N at%] | mean abs diff [15N at%] | cum diff [15N at%] | mean abs diff [15N at%] | cum diff [15N at%] | mean abs diff [15N at%] |
| H+M        | 99                   | 7.8                  | 107                  | 6.1                  | -8                    | 2.3                    |
| I+I        | 1                    | 6.3                  | -14                  | 5.3                  | 15                    | 3.4                    |
| H+I        | 53                   | 4.2                  | 18                   | 3.0                  | 37                    | 2.4                    |