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USE OF ISOTOPES FOR IDENTIFICATION OF N_2O SOURCES FROM SOILS

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Abstract
Natural processes and human activity play a crucial role in altering the nitrogen cycle and increasing nitrogen oxide (N_2O) emissions. Nitrous oxide isotopes ^{15}N and ^{18}O are important parameters that can help to explain the sources of N_2O gas, as well as their circulation under different soil physical properties. The main goal of the study is to analyze the possibilities of using dinitrogen isotopes ^{15}N and ^{18}O, measured in soil samples, for the identification of N_2O sources. A total of 16 plots were sampled. Each soil sample was assigned a code. Wetting of the samples was carried out to create wet aerobic conditions and wet anaerobic conditions. N_2O measurements were performed in laboratory conditions using the Picarro G5131-i device. The $^{15}N\alpha$ and $^{15}N\beta$ values obtained in the measurement data were used to calculate the $\delta^{15}N^{SP}$ and $\delta^{15}N^{bulk}$ values. The obtained $\delta^{15}N^{SP}$ and $\delta^{15}N^{bul}$ values were analysed using two methods – descriptive statistics and Kruskal-Wallis test. The test showed that there are statistically significant differences between $\delta^{15}N^{SP}$ values (p-value <0.0001), and $\delta^{15}N^{bulk}$ there was no significant difference (p-value 0.885).
Key words: N_2O isotopes, soil tillage, Picarro G5131-i.

Introduction

In order for life to form and exist, several important processes take place in nature, which are based on various elements, their mutual interaction and influencing factors. One such element is nitrogen (N). Although nitrogen is the most abundant element in the atmosphere and approximately 78% of the atmosphere is nitrogen gas (N_2), most living organisms cannot use nitrogen in this way. Therefore, through various processes, for example, during the nitrogen cycle, nitrogen fixation takes place, the result of which is the formation of several complex organic compounds that are necessary for living organisms. In order to form the necessary nitrogen compounds, various compounds of nitrogen elements enter both the atmosphere and the hydrosphere as by-products in the circulation cycle, which can have a negative impact on the surrounding environment and climate. One of the compounds that have a negative impact on the climate is N_2O gas – nitrous oxide.

The importance of atmospheric N_2O in the global environment is divided into two distinct features. This property is that N_2O regulates stratospheric ozone. It is a consequence of the reaction of N_2O in the stratosphere with $O(^1D)$ radicals in the following reaction $O(^1D) + N_2O \rightarrow 2\ NO$ or $N_2 + O_2$. The reaction pathway for NO, which occurs in about 60% of cases, is the dominant source of stratospheric NO. NO participates in several catalytic cycles that contribute to the destruction of ozone. In this connection, N_2O affects several properties of the stratosphere and indirectly regulates the amount of ultraviolet radiation reaching the earth. Another important factor is that N_2O is a strong greenhouse gas. This is mainly due to its strong multi-infrared band from 7.7 to 17 mm and its long chemical lifetime of about 130 years (Brenninkmeijer & Röckmann, 1999). Nitrous oxide has 300 times the global warming potential of nitrogen oxide and plays a major role in the depletion of stratospheric ozone (Butterbach-Bahl *et al*., 2013).

Human activity has contributed significantly to global warming by increasing concentrations of greenhouse gases such as nitrogen dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4). Of all anthropogenic sources of greenhouse gases, agricultural activity accounts for approximately 13% of all emissions (Abdalla *et al*., 2014).

Soils are the main source of the greenhouse gas (GHG) N_2O, accounting for about 69% of the anthropogenic atmospheric load of this gas. N_2O production is mainly biological and occurs through nitrification (oxidation of ammonia and denitrification of the nitrifier) and dissimilation of nitrates (denitrification and ammonitrification) (Figure 1) (Marinas *et al*., 2010).

GHG emissions from soil are caused by both microbial activity and the chemical processes of root respiration occurring in the soil. Emission flux rates are strongly influenced by soil moisture, soil temperature, nutrient availability and pH values, as well as crop related parameters (Chapuis-lardy *et al*., 2007). Meteorological and climatic parameters, as well as land use information, are very important for estimating GHG emissions from soil. The main factors affecting soil GHG emissions, including N_2O, have been identified (Figure 2).

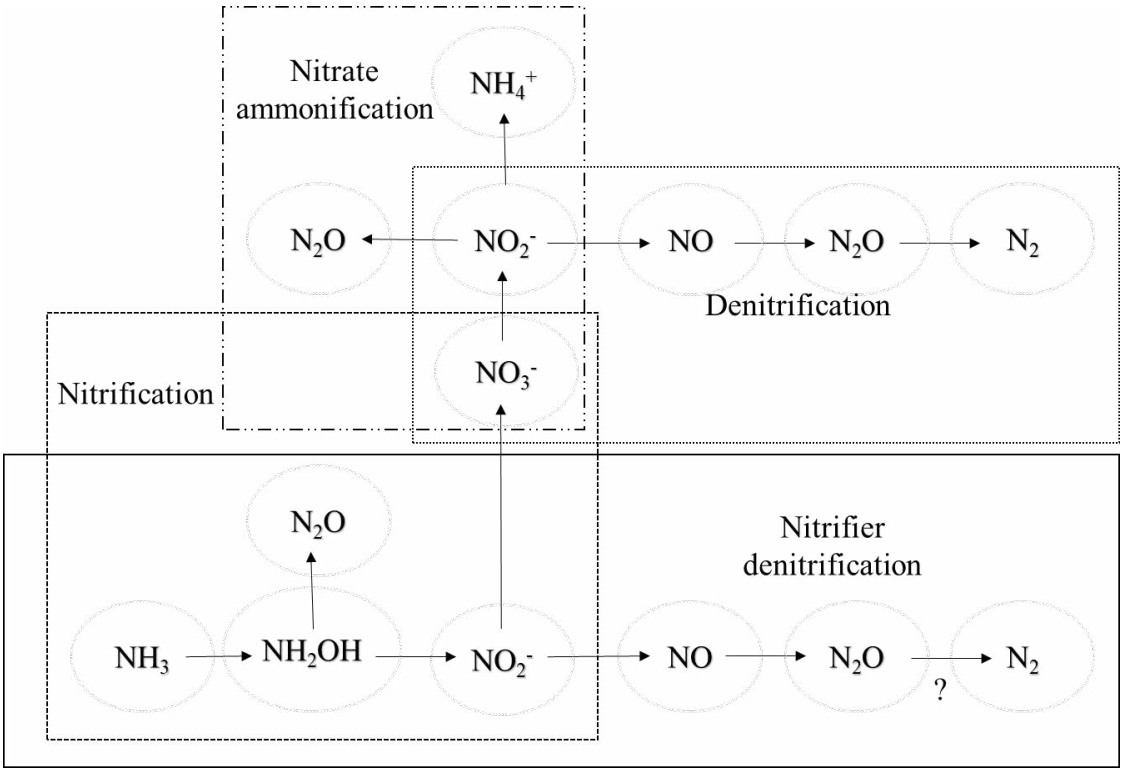

Figure 1. Microbial sources of N_2O in soil (Created by the authors after Marinas *et al*., 2010).

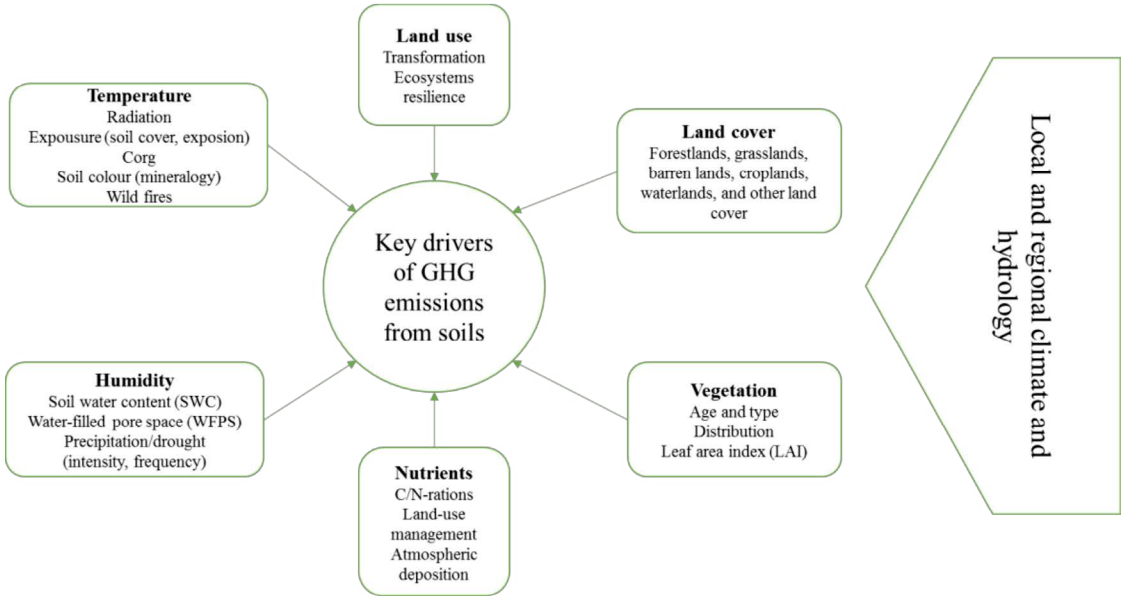

Figure 2. Key drivers of greenhouse gas (GHG) emissions from soil (Created by the authors after Oertel *et al*., 2016).

Minimum tillage and no-till systems have become widespread in various agricultural regions, almost completely replacing traditional tillage systems in North and South America (Alvarez & Steinbach, 2009). The benefits of reduced tillage compared to traditional methods include reduced diesel and energy consumption and higher productivity. Water infiltration is improved and soil erosion is reduced as plant residues are left on the field (Holland, 2004). Under the right conditions, reduced tillage can improve yields and improve energy and resource efficiency.

However, reduced tillage, especially on loam and clay soils, and in cool and wet conditions can reduce

soil aeration and stimulate N_2O emissions that offset the CO_2 reduction effect (Rochette, 2008).

Since climate change is a hot topic not only now, but in the long term, various important studies related to this atmospheric change have been conducted. Most studies related to GHG emissions from the soil are based on the physical properties of the soil – soil temperature, moisture, pH level, as well as the main microbiological processes – nitrification and denitrification. These studies have contributed greatly to understanding the global cycle of nitrous oxide. However, the study of GHG emissions is not limited to this. The role of isotopes in the study of various processes is increasingly emphasized (Zhu *et al*., 2019). Thus, nitrous oxide isotopes ^{15}N and ^{18}O are an important parameter that can help trace the sources of N_2O gas and its circulation in certain processes occurring in the soil.

Materials and Methods

Soil samples collected from 28 experimental fields were used for this study. 12 of fields are managed organically and 16 fields are managed conventionally. Two types of tillage were used for the fields – reduced tillage and conventional tillage. Experimental fields are located near Talsi town in Stende breeding and Research and Study farm of Latvia University of Life Sciences and Technologies 'Pēterlauki' (Figure 3) near Jelgava city (Figure 4).

Soil samples were taken at five locations from the field to obtain one disturbed soil sample (Figure 3, 4). This method was used because the composition of the soil within the field may vary.

Figure 3. Sampling fields points on the map at Stende Research Centre.
Soil samples were taken at five field locations at diagonal intersections, from which one mixed soil sample was created.

Figure 4. Sampling fields points on the map at Research and Study farm 'Pēterlauki'.
Soil samples were taken at five field locations in the corners of the field and in the middle of the field, from which one mixed soil sample was created.

The samples were weighed in 3-liter buckets, each bucket containing 1.5 kg of soil (Figure 5). For each experimental field, two soil samples were created, which were provided with different moisture conditions – one wet aerobic conditions and the other wet anaerobic conditions. Rainwater was used to moisten the soil samples, the samples were moistened once every three days – 150 mL of water was used for one sample and 300 mL of water for the other samples. The amount of water required for wetting the soil was calculated taking into account the weight of the soil before and after wetting and evaporation were calculated. The study was conducted in laboratory conditions.

N_2O measurements were performed in laboratory conditions using the Cavity Ring Down Spectroscopy (CRDS) device Picarro G5131-i device (Picarro, Inc., California, USA). The CRDS device Picarro G5131-i

Figure 5. Three liter buckets with mixed soil samples connected with CRDS device Picarro G5131-i.

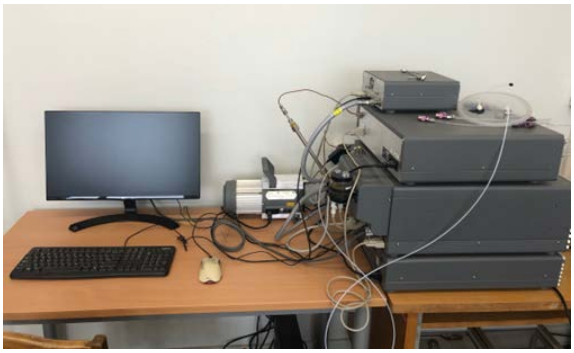

Figure 6. Isotope and gas concentration analyser CRDS device Picarro G5131-i.

Isotope and Gas Concentration Analyser enables simultaneous site-specific and bulk measurements of $\delta^{15}N$ and $\delta^{18}O$ in N_2O (Figure 6). It is an ideal solution to identify and measure the source of N_2O emissions by measuring in the laboratory. N_2O isotopes can be used to identify sources in the global nitrogen cycle by identifying nitrification and denitrification processes in soils. The G5131-i analyser measures $\delta^{15}N$, $\delta^{15}N\alpha$ and $\delta^{15}N\beta$ with an accuracy of 0.7‰, $\delta_{18}O$ with an accuracy of 0.7‰ and N_2O concentration with an accuracy of <0.05 ppb (all accuracy measurements are averaged over 10 minutes).

Each soil sample was assigned a code such as B_BA_O_1 and B_C_O_CT_1, B for organic farming type, BA for cultivated crop, in this sample forests, O for soil type, in this case organic soil, CT for tillage, in this case conventional tillage and numbers 1 and 2 denote humidity mode, 1 – normal humidity conditions, 2 – over humid conditions. These two codes are assigned to samples collected from the same field (Table 1).

The $^{15}N\alpha$ and $^{15}N\beta$ values obtained in the measurement data were used to calculate the $\delta^{15}N^{SP}$ and $\delta^{15}N^{bulk}$ values. Such a calculation is necessary for a more in-depth evaluation of the connection of isotopes with the microbiological processes taking place in the soil and to compare them with the existing research results. Calculation examples are shown in Equations 1 and 2, the data used, as well as the results, can be seen in Table 1.

$$\delta^{15}N^{SP} = \delta^{15}N^{\alpha} - \delta^{15}N^{\beta} \quad (1)$$

$$\delta^{15}N^{bulk} = \frac{\delta^{15}N^{\alpha} + \delta^{15}N^{\beta}}{2} \quad (2)$$

Table 1

Example of data matrix from Picarro G5131-i

Date	Sample	Chamber	$^{15}N^{\alpha}$, ‰	$^{15}N^{\beta}$, ‰	$\delta^{15}N^{SP}$, ‰	$\delta^{15}N^{bulk}$, ‰
18.08.2020	B_C_O_CT_1	1	3.90	2.53	1.37	3.22
		2	2.38	3.42	-1.04	2.90
		3	-0.47	4.34	-4.81	1.94

Notes: B – organic farming type; C – clover; O – organic soil; CT – conventional tillage; 1 – normal humidity conditions.

Using the calculated $\delta^{15}N^{SP}$ values, it is possible to determine and analyse microbiological processes in the soil, nitrification and denitrification can be determined. To date, several studies have been carried out on the use of N_2O isotopes for the determination of nitrification and denitrification, as well as for the observation of microbiological processes. Based on (Sutka *et al.*, 2006) the average values of studies for nitrification in soil are $\delta^{15}N^{SP}$ 33 ‰ and $\delta^{15}N^{bulk}$ – 0.9‰, while the values of denitrification are $\delta^{15}N^{SP}$ 0.1‰ and $\delta^{15}N^{bulk}$ – -23 ‰.

The obtained $\delta^{15}N^{SP}$ and $\delta^{15}N^{bulk}$ values were analysed using two methods – descriptive statistics and Kruskal-Wallis test. The Kruskal Wallis test is a non-parametric alternative to One Way ANOVA. Non-parametric means that the test does not assume that data comes from a particular distribution. The test determines whether the medians of two or more groups differ. Like most statistical tests, it calculates the test statistics and compares it to the cutoff point of the distribution. The test statistics used in this test is called the H statistics. The Kruskal Wallis test indicates if there is a significant difference between the groups.

Results and Discussion

In the conventional tillage fields, the maximum value of $\delta^{15}N^{SP}$ was 18.58‰, the minimum value is -53.41‰, and the maximum value of $\delta^{15}N^{bulk}$ was 16.28‰, the minimum value is -56.97‰. In the fields where reduced tillage was performed, the maximum value of $\delta^{15}N^{SP}$ is 14.34‰, the minimum value is -36.91‰, while the maximum value of $\delta^{15}N^{bulk}$ is 26.76‰, and the minimum value was -56.77‰.

In the table of descriptive statistics (Table 2), it is shown that there were more conventional tillage fields than reduced tillage, so conventional tillage data could be more accurate than reduced tillage. The lowest $\delta^{15}N^{SP}$ value is conventional tillage, but $\delta^{15}N^{bulk}$ minimum values are very close in both types of tillage. The highest $\delta^{15}N^{SP}$ value is conventional tillage. Mean and median values for $\delta^{15}N^{SP}$ values are lower than for bulk values.

Table 2

Descriptive statistics (Quantitative data) of N2O $\delta^{15}N^{SP}$ and $\delta^{15}N^{bulk}$ values by conventional tillage and reduced tillage

Statistics	$\delta^{15}N^{SP}$ | CT	$\delta^{15}N^{SP}$ | RT	$\delta^{15}N^{bulk}$ | CT	$\delta^{15}N^{bulk}$ | RT
No. of observations	1188	324	1188	324
No. of missing values	0	0	0	0
Minimum, ‰	-53.408	-36.906	-56.973	-56.766
Maximum	18.579	14.342	16.279	26.758
1st Quartile	-9.052	-15.369	0.465	2.100
Median	-3.313	-7.542	5.082	4.483
3rd Quartile	2.074	-0.307	8.362	7.569
Mean	-4.086	-7.818	0.275	3.332
Variance (n-1)	75.841	106.624	198.168	126.629
Standard deviation (n-1)	8.709	10.326	14.077	11.253

Analysing the values of $\delta^{15}N^{SP}$ depending on the type of tillage, it can be seen that the largest amplitude is reduced tillage, while the amplitude of conventional tillage is smaller, and more extreme values and outliers are visible in conventional tillage. Median value for conventional tillage are -3.313‰, but for reduced tillage -7.542‰. Mean value for conventional tillage is -4.086‰, but for reduced tillage -7.818‰. We can see that mean values are lower for bot soil tillage's (Figure 7).

Analysing the values of $\delta^{15}N^{bulk}$ according to the type of tillage, it can be seen that the largest amplitude is conventional tillage, while the smaller amplitude is reduced tillage. Maximum values and outliers are significantly more in conventional tillage, but less in reduced tillage. Median value for conventional tillage are 5.082‰, but for reduced tillage 4.483‰. Mean value for conventional tillage is 0.275‰, but for reduced tillage 3.332‰. We can see that mean values are lower for both types of soil tillage (Figure 8).

The obtained $\delta^{15}N^{SP}$ and $\delta^{15}N^{bulk}$ values were analysed using two methods – descriptive statistics and Kruskal-Wallis test. The test showed that there

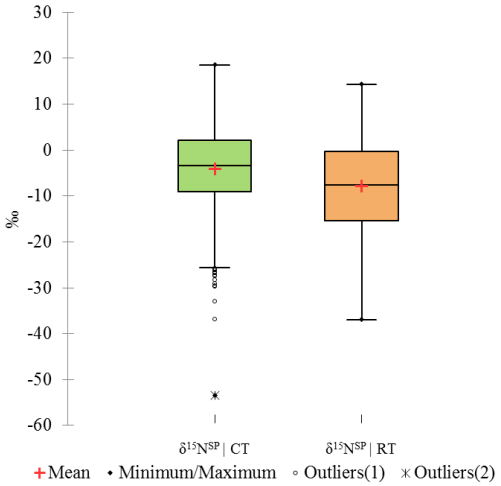

Figure 7. The value of N_2O $\delta^{15}N^{SP}$ distribution by conventional tillage (CT) and reduced tillage (RT).

are statistically significant differences between $\delta^{15}N^{SP}$ values (p-value <0.0001), and $\delta^{15}N^{bulk}$ there was no significant difference (p-value 0.885).

In the past, studies of N_2O GHG emissions from soil have also been conducted, and these studies have

addressed several factors that should be taken into account.

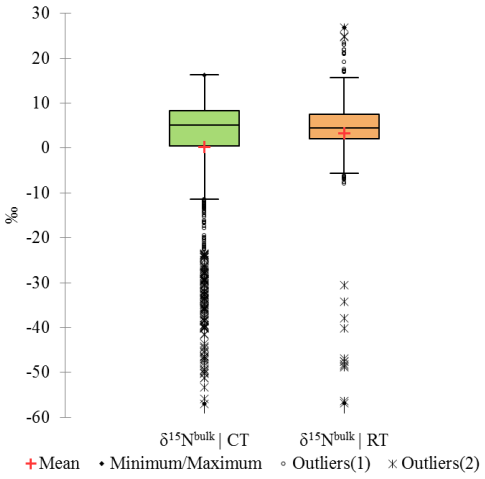

Figure 8. The value of N_2O $\delta^{15}N^{bulk}$ distribution by conventional tillage (CT) and reduced tillage (RT).

In (Zalite *et al*., 2021) research, clay soil and GHG emissions were addressed. GHG emissions from clayed soils by crop groups, the Kruskal-Wallis test shows a statistically significant difference in the effect of cultivated crops on GHG emissions. N_2O emissions showed a statistically significant difference between crop groups. This indicates that the type of soil is also a very important factor affecting greenhouse gases that should be taken into account.

Conclusions

1. The CRDS device Picarro G5131-i is successfully used to measure N_2O isotopes in soil under laboratory conditions, which provides an in-depth study of N_2O.
2. N_2O is an isotopically complex molecule, but isotopes of this molecule and $\delta^{15}N^{bulk}$ and $\delta^{15}N^{SP}$ are valuable for tracking soil N_2O emissions and microbiological processes.
3. The obtained $\delta^{15}N^{SP}$ and $\delta^{15}N^{bulk}$ values were analysed using two methods – descriptive statistics and Kruskal-Wallis test. The test showed that there are statistically significant differences between $\delta^{15}N^{SP}$ values (p-value <0.0001), and $\delta^{15}N^{bulk}$ there was no significant difference (p-value 0.885).
4. The calculated data for $\delta^{15}N^{bulk}$ and $\delta^{15}N^{SP}$ indicate consistent results, with some exceptions, as well as their changes over time. However, it does not give a clear picture of the microbiological processes of the soil under the relevant conditions.
   Median value for conventional tillage are -3.313‰, but for reduced tillage -7.542‰. Mean value for conventional tillage is -4.086‰, but for reduced tillage -7.818‰.

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