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Contrasting isotopic responses of dryland and wetland plants to a century of global anthropogenic changes in nutrient cycling

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Abstract. Anthropogenic emissions of carbon dioxide and reactive nitrogen in various forms disrupt the functioning of ecosystems around the world. In Europe, many valuable habitats, particularly wetlands and semi-natural dry grasslands, are under threat from ongoing eutrophication. However, due to contrasting water regimes, the uptake of anthropogenic nitrogen by plants in these ecosystems is different and is also interrelated with an increase in trophic level in both habitats.

In our study, we measured the $\delta^{15}N$ and $\delta^{13}C$ values, as well as the total nitrogen content (TN), of 99 pairs of foliar samples collected from seven species of vascular plants in both dry grasslands and wetlands in Poland. Each pair consisted of a historical sample collected from a herbarium voucher dating from before 1939 (i.e. before artificial fertilisers were widely used in agriculture) and a contemporary sample collected in 2024 from the same species in a similar location.

We performed t-tests to determine whether there were significant differences in the means of $\delta^{15}N$, TN and $\delta^{13}C$ between samples from the two different habitats. Next, we calculated the differences in $\delta^{15}N$, TN and $\delta^{13}C$ between the contemporary and historical samples for each pair. We then tested whether the difference for each species and habitat type was significantly different from zero using 90% confidence intervals. Using multiple linear regression, we analysed the relationships between differences in $\delta^{15}N$ and TN over time and the following factors: habitat type, the proportion of farmland in the surrounding landscape, the consumption of synthetic nitrogen fertiliser, and NOx deposition.

$\delta^{15}N$ and TN values were lower in dry grassland species than in wetland species, in both the contemporary and historical subsets. For dry grassland species, the mean $\delta^{15}N$ value was lower in contemporary samples than in historical ones. For wetland species, the opposite was true. The difference in $\delta^{15}N$ values between pairs of samples was positively related to the amount of farmland in the surrounding landscape. The mean TN was higher in contemporary than in historical wetland samples, but not in dry grassland plants. The mean $\delta^{13}C$ value, corrected for the Suess effect, was lower in contemporary samples than in historical ones. The mean difference was −0.51 ‰ for dry grassland and −3.85 ‰ for wetland species.

Our study revealed that the century of fossil fuel-derived carbon emissions, increased nitrogen input into the environment, and dominance of artificial fertilisers and combustion-derived nitrogen over biological nitrogen sources have not led to consistent responses across habitats and species. While the isotopic composition of nitrogen and carbon in plant tissues in Central Europe has undoubtedly changed, this change is highly context-dependent. Its magnitude and direction are impacted by the type of habitat and the identity and/or ecology of the species. As expected, man-made alterations appear to be more pronounced in wetland environments than in dryland habitats. Furthermore, the source of disruption may differ between the two habitat types. Specifically, wetlands are exposed to a multitude of anthropogenic nitrogen and carbon sources, whereas dry grasslands seem to be predominantly affected by changes in the composition of the atmosphere.

1 Introduction

Disruption to the global nitrogen and carbon cycles caused by human activities is one of the major threats to the functioning of the biosphere (Hessen et al., 1997; Richardson et al., 2023). The main source of anthropogenic carbon is the combustion of fossil fuels, which produces carbon dioxide (CO_2), one of the main greenhouse gases. However, the high-temperature combustion of fossil fuels in the atmosphere (e.g. in engines of cars and trucks and in electrical power generation plants) produces also nitrogen oxides (NO and NO_2), which play role in the formation of both smog and acid rain, and contribute to the atmospheric nitrogen deposition to the ecosystems (mostly after conversion to nitric acid HNO_3, Seinfeld and Pandis, 2006; Hertel et al., 2012). Fossil fuel combustion began on a massive scale in the Industrial Era (Pirani, 2018). Later, during the 20th century and continuing to the present day, anthropogenic nitrogen input has increased, particularly due to the invention of an efficient method of synthesising ammonium through fixation of atmospheric nitrogen N_2 (Bateman and Kelly, 2007; Bodirsky et al., 2012). This process enabled the large-scale production and use of artificial fertilisers in agriculture, which was one of the foundations of the so-called Green Revolution of the 1960s. (Bodirsky et al., 2012). However, excess of anthropogenic, bioavailable nitrogen input into natural ecosystems usually leads to profound changes in their productivity, biodiversity and functioning, and often cannot be easily reversed (Stevens et al., 2004; Dise et al., 2011; Van der Plas et al., 2024). Furthermore, the combined effect of increased CO_2 and reactive nitrogen may have a more negative impact on biodiversity and ecosystem functioning than the effects of each factor acting alone (Bakker et al., 2024; Reich et al., 2024).

An excess of nitrogen input is a well-recognised threat to a wide range of ecosystems that are more susceptible to eutrophication, particularly aquatic habitats (Schindler, 2006), but also species-rich semi-natural grasslands in Europe (Stevens et al., 2004; Bobbink et al., 2010; Dise et al., 2011). Eutrophication in aquatic ecosystems, mainly caused by nitrogen and phosphorus, leads to uncontrolled algal or cyanobacterial blooms. These blooms can result in episodes of hypoxia (low oxygen levels), reduced light penetration, the release of toxic compounds and unpleasant odours, increased water turbidity, and alterations to biocoenoses. (Schindler, 2006; Erisman et al., 2013). These changes can include the extinction of underwater plants (Qin et al., 2013) and increased fish mortality (Schindler, 2006). In grassland ecosystems, high nitrogen input may lead to changes in nutrient and water cycling, soil acidification and increased biomass production (Bobbink et al., 2010; Meier et al., 2022). Higher productivity can lead to the dominance of fast-growing, expansive species, while the production of large amounts of litter can inhibit the growth of smaller, less competitive, and often rare species (Meier et al., 2022; Chen et al., 2023). Larger biomass production and litter accumulation also reduce the amount of light reaching ground level, which usually harms germination and seedling survival (Ruprecht et al., 2010; Meier et al., 2022).

It is estimated that in 2020, the total amount of nitrogen produced globally by humans was 226 Tg N/year, while natural nitrogen fixation ranged from 58 to 128 Tg N/year (Galloway et al., 2021). It is estimated that global consumption of artificial nitrogen fertilisers increased nearly tenfold from 11.3 Tg N/year in 1961 to 108 Tg N/year in 2022 (Lu and Tian, 2016; FAO, 2024). The highest use of nitrogen-based fertilisers in agriculture was among the European Union's main agricultural producers, particularly in France (1.7 million tonnes), Poland and Germany (1.0 million tonnes each) (Eurostat, 2025). In Poland in 2020, the average use of artificial nitrogen fertilisers was 70.4 kg per hectare of agricultural land (GUS, 2020). As a result, Poland can be classified as one of the countries with the highest artificial fertiliser consumption per hectare of agricultural land, with fertilisation levels exceeding the EU average by about one third (Piwowar, 2022). It is estimated that only about 30-50 % of the nitrogen from the fertilisers applied is taken up or assimilated by crops, with the remainder being lost through leaching, surface runoff and gaseous emissions (Lehnert, 2015; Choi et al., 2017).

The physiology and evolutionary strategy of nitrogen uptake by plants, such as through mycorrhizae or the presence of nitrogen-fixing bacteria, influences the ratio of stable nitrogen isotopes, i.e. $^{15}N/^{14}N$ ($\delta^{15}N$). Usually, plants discriminate against ^{15}N, resulting in lower $\delta^{15}N$ values in plant tissue than in their N source (Dawson et al., 2002). But the main driver of variation in $\delta^{15}N$ is the type and availability of nitrogen sources and their isotopic composition (Evans, 2001). Changes in the $\delta^{15}N$ of plant-available nitrogen are typically mirrored in plant tissue values. Therefore, stable isotopes are a useful tool to trace anthropogenic nitrogen input (Kendall et al., 2007; Hastings et al., 2013). There are three major sources of human-derived nitrogen with distinct $\delta^{15}N$ signatures: 1) Synthetic fertiliser: widely used since the 1950s, produced via the Haber–Bosch process, has $\delta^{15}N$ values close to air N_2 or slightly negative ($\delta^{15}N$ between -4 ‰ – 0 ‰) (Bateman and Kelly, 2007). 2) Organic nitrogen from untreated wastewater and manure (also in the form of atmospheric deposition of agricultural NH_3). Volatilisation and microbial transformations of this type of nitrogen come with strong ^{14}N loss, giving the remaining nitrogen high $\delta^{15}N$ values (approx. 5 ‰ – 20 ‰) (Bateman and Kelly, 2007). This isotopic enrichment has been used, e.g. to authenticate products of certified organic farming (Muñoz-Redondo et al., 2023) or to trace the historical use of manure in farming (Halvorsen et al., 2023). 3) Atmospheric deposition from fossil fuel combustion in power plants and engines. The $\delta^{15}N$ of reactive nitrogen from fossil fuel combustion is generally negative but can be variable due to atmospheric transformations (Elliott et al., 2019), soil microbial processing and evolutionary strategies of plants (Yao et al., 2011).

Nitrogen content (relative to other elements) in plant tissues is a species-specific trait, with species with competitive and ruderal strategies often being more N-rich and species which are stress-tolerant and have strongly lignified tissues being N-poor (Tilman and Wedin, 1991; Tian et al., 2019). However, it has been proven that the relative content of nitrogen in relation to other macronutrients, but also the nitrogen concentrations in the tissues, could be dependent on nitrogen availability in the environment (Palpurina et al., 2019). Moreover, under elevated CO_2, the nitrogen concentration in plant tissues tends to decrease (Cotrufo et al., 1998), which can be a result of multiple mechanisms, including the simple dilution effect but also changes in root architecture (Taub and Wang, 2008).

Stable carbon isotopes ($\delta^{13}C$) in plant biomass can be used to assess long-term changes in carbon cycling, photosynthetic conditions, and environmental stressors affecting plant life (Cernusak et al., 2013). Carbon isotopic signatures in terrestrial plants reflect the values of CO_2, so studying historical samples, including plant biomass, provides evidence for the Suess effect: the progressive depletion of atmospheric ^{13}C due to fossil fuel emissions (Keeling, 1979). On a much smaller level, changes in CO_2 concentration also affect ^{13}C discrimination (Keeling et al., 2017). Photosynthesis in general is a process strongly discriminating against ^{13}C, but the magnitude of this discrimination depends on plant physiology, especially the type of photosynthesis (C3/C4) (Farquhar et al., 1989). Within one type of photosynthesis, $\delta^{13}C$ is altered by stomatal activity: long periods of closed stomata (higher water-use efficiency) mean that the plant is using a limited CO_2 pool, resulting in lesser discrimination and higher $\delta^{13}C$ values (Seibt et al., 2008). Aquatic plants can use dissolved organic carbon (DIC) for photosynthesis; in fully submerged plants, it is the only carbon source. As opposed to atmospheric CO_2, $\delta^{13}C$ of DIC (and aquatic plants) undergo various site-dependent changes in both directions, depending on the source of carbon, type of water body (Pronin et al., 2024), water chemistry - mainly pH (Thompson et al., 2001) and abundance of DIC (Schulte et al., 2011; Chappius et al., 2017). This variety of factors determines the final value of $\delta^{13}C$, making the practical use of carbon isotopes as a marker of eutrophication in wetland ecosystems comparable to the use of nitrogen isotopes (Wang et al., 2015; Hereida et al., 2022).

A couple of studies have already used historical plant material stored in herbaria to track changes in the isotopic and chemical composition of plant biomass since the 19th century (Peñuelas and Filella, 2001; McLauchlan et al., 2010; Alldred et al., 2024; Kühn et al., 2025) or even the 18th century (Peñuelas and Matamala, 1990). Some of these studies have identified similar trends; for example, a decrease in $\delta^{15}N$ was observed in foliar samples during the 20th century, as demonstrated for prairie plants in Kansas (McLauchlan et al., 2010) and Mediterranean terrestrial plants in north and east Spain (Peñuelas and Filella, 2001). However, McLauchlan et al. (2010) indicated that the main reason for this trend was an increase in atmospheric CO_2 levels (and thus stronger N-limitation) thought, Peñuelas and Filella (2001) attributed it to higher anthropogenic nitrogen input into ecosystems. Furthermore, McLauchlan et al. (2010) found that leaf nitrogen content decreased over the 20th century, while Kühn et al. (2025) observed an opposite trend in vascular plants from mesic grasslands and crop fields in Germany, a country with a similar, to our study area, intensive and crop-based agricultural sector (Eurostat, 2025) described below. It is also worth noting that most of the aforementioned studies focused on terrestrial habitats, while recent study by Alldred et al. (2024) also found herbarium vouchers of macroalgae to be a very promising source of information in studying past biogeochemical trends in water environments.

To date, no studies have attempted to compare at the same time different habitat types that may be exposed to varying levels of anthropogenic nitrogen. Similarly, no studies have analysed the correlation between the magnitude of change and local land use factors. Considering the above discrepancies and gaps in knowledge, we formulated the following hypotheses to verify in our study:

1) Wetland plants and dry grassland plants exhibit different patterns of shifts in stable isotope composition.
2) Effects of eutrophication are more visible in wetlands, especially in submerged species, which show a drop in $\delta^{13}C$ and an increase in $\delta^{15}N$.
3) TN in plant tissues increased over the last century.

4) The change in TN and $\delta^{15}N$ is correlated with the proportion of agricultural areas' consumption of artificial nitrogen fertilisers in agriculture, and the atmospheric nitrogen deposition in the modern landscape.
5) $\delta^{13}C$ of dry grassland plants decreased by about 1.5 ‰ over the last century due to the Suess effect.

2 Methods

2.1 Selection of species and specimens for analyses

For our study, we selected species that had to be represented by well-preserved and well-developed herbarium vouchers in the Herbarium of the Faculty of Biology, University of Warsaw (WA). Those herbarium vouchers should be collected before 1939, so before the artificial fertilisers became widespread in Central Europe (Merta-Staszczak and Zwyrzykowska-Wodzińska, 2017; Tian et al., 2022), from at least six locations that could be linked to contemporary occurrences of the species. We considered a species to be currently present at a given location (i.e. appropriate to the location of the herbarium specimen): i) if we had personally observed the species there in the last decade, ii) if we could identify patches of potential habitat of the species on ortophotographs (available at https://www.geoportal.gov.pl/), or iii) if we found observations of the species reported in recent botanical literature or in iNaturalist (https://www.inaturalist.org/).

To reduce the impact of various potential nitrogen sources on the chemical composition of plant biomass, we excluded species that fix nitrogen via symbiotic bacteria. We chose plant species from taxa which are known to be non-mycorrhizal (*Caryophyllaceae*, *Brassicaceae*, *Hydrocharitacae*, *Potamogetonacea*, *Nymphaea alba*) or weakly associated with fungi (especially in wetland habitats) and if so, mostly of AM mycorrhizal type through which they obtain rather phosphorus than nitrogen (*Alismataceae*, genus *Carex*) (George et al., 1995; Miller et al., 1999; Cornwell et al., 2001; Unger et al., 2016). Our selection included relatively common species in two habitat types: dry grasslands and wetlands (springs, lakes and rivers) (see Table 1). The stability of moisture conditions in these habitats (often deficient in grasslands and always abundant in wetlands) helped to reduce the impact of variation in water availability at the time of material collection on the results. Finally, we selected 14 vascular plant taxa, two of which were aggregations of two closely related species (Table 1).

Table 1: Taxon characteristics and number of paired samples collected from historical and appropriate contemporary specimens. Species nomenclature follows World Flora Online (2025).

Species/genus name	Family	Habitat type	Number of sample pairs	Remarks
Alisma spp.	*Alismataceae*	wetlands	8	*Alisma plantago-aquatica* L. (7 pairs of samples) and *Alisma lanceolatum* With. (1 pair of samples)
Arabis hirsuta (L.) Scop.	*Brassicaceae*	dry grasslands	7	-
Cardamine amara L.	*Brassicaceae*	wetlands	7	-
Carex caryophyllea Latourr.	*Cyperaceae*	dry grasslands	7	-
Carex flacca Schreb.	*Cyperaceae*	dry grasslands	5	-
Carex paniculata L.	*Cyperaceae*	wetlands	4	-
Carex praecox Schreb.	*Cyperaceae*	dry grasslands	8	-
Dianthus carthusianorum L.	*Caryophyllaceae*	dry grasslands	10	-
Elodea canadensis Michx.	*Hydrocharitaceae*	wetlands	7	-
Erysimum spp.	*Brassicaceae*	dry grasslands	5	*Erysimum odoratum* Ehrh. (4 pairs of samples) and *Erysimum hungaricum* Zapał. (1 pair of samples)
Nymphaea alba L.	*Nymphaeaceae*	wetlands	7	-
Sagittaria sagittifolia L.	*Alismataceae*	wetlands	8	-
Silene otites (L.) Wibel	*Caryophyllaceae*	dry grasslands	9	-
Stuckenia pectinata (L.) Börner	*Potamogetonaceae*	wetlands	8	-

2.2 Study area

The spatial arrangement of our study was predominantly driven by the origin of the herbarium specimens of the selected species. As Warsaw was part of the Russian Empire until the First World War, most of the materials stored in the herbarium from before that period were collected within its former borders, except in the vicinity of the Tatra Mountains, which was a popular destination among botanists at that time. We excluded vouchers collected outside the current Polish borders. To avoid oversampling certain popular areas, we did not include in our study samples of the same species collected less than a few kilometres apart. Finally majority of the selected pairs originate from the eastern half of Poland. According to the Köppen-Geiger climate classification, the region predominantly has a warm temperate, fully humid climate with a warm summer (Cfb) (Kottek et al., 2006). The mean annual temperature of the sampled locations varied from 6.35 °C to 9.05 °C (mean 8.26 °C), while annual precipitation ranged from 496 mm to 1187 mm (mean 632 mm). The mean temperature of the coldest month (January) ranged from −3.75 °C to −0.15 °C (mean −2.01 °C), while the mean temperature of the warmest month (July) ranged from 16.15 °C to 19.45 °C (mean 18.67 °C) in the period 1981–2010 (Karger et al., 2017).

2.3 Sampling of plant material

Each herbarium voucher sample consisted of two to five leaf fragments collected from one or several individuals, depending on the availability of material and the state of preservation of the specimens. According to information obtained from the head of the WA Herbarium, the specimens stored there were not prepared or preserved using nitrogen-containing substances. We attempted to obtain contemporary samples in the same locality as the historical one. The accuracy of relocation was related mostly to the level of detail provided in the herbarium voucher description. If the original location description was precise (it allowed for site relocation with uncertainty less than one kilometre), we searched for the species in the same location, and if we were unable to find it there, then we searched in a radius of a maximum of 10 km from the original site. However, if the description was very general (e.g. pointing to a large town), we looked for the focal species in the broader vicinity of that town in suitable habitats. The total number of samples collected was 198, comprising 99 historical samples collected between 1855 and 1938 (median 1905) and 99 contemporary samples that were paired with their respective locations. All contemporary samples were collected between April (for the early-flowering species as *Arabis hirsuta* and *Erysimum* spp.) and September (for some of the aquatic plants) of 2024. Each contemporary sample, similarly to the historical ones, consisted of two to five leaf fragments collected from several individuals per locality (Fig. 1). We selected fully developed, green and healthy plants that were mostly in the flowering or early fruiting phase. Additionally, two to three specimens from most of the currently sampled populations we collected and deposited as herbarium vouchers in the Herbarium WA for future reference.

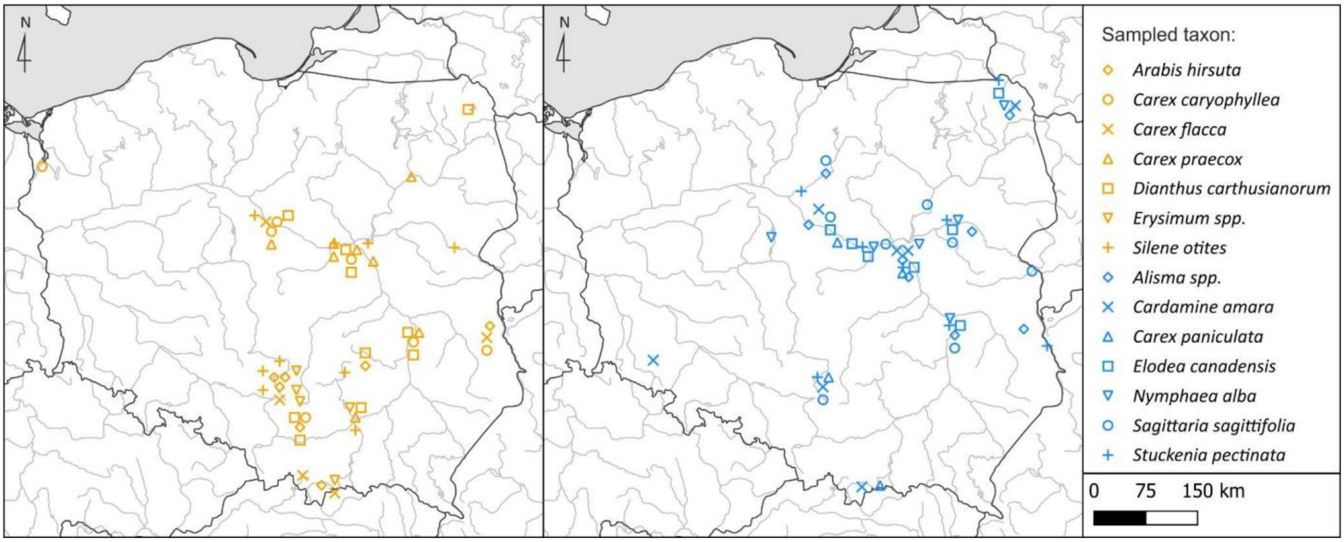

Figure 1: Localities of the sampled populations of dry grassland species (on the left) and wetland species (on the right). Overlapping locations were randomly dispersed to increase visibility (spatial data source: Bohn et al., 2001-2003).

2.4 Laboratory analyses

Fresh samples were dried in 40 °C for 24 h within one day after collecting. Each sample was homogenized by crushing in a pestle and mortar or cut into small pieces using scissors. Two subsamples were weighted into tin capsules – approx. 5 mg for quantitative analysis and 3 mg for stable isotope analysis. Total carbon and nitrogen were measured using Thermo Flash 2000 Elemental Analyzer with TCD detector. Stable carbon and nitrogen were measured in a Thermo Delta V Plus Isotope Ratio Mass Spectrometer coupled with a Flash 2000 Elemental Analyser. Stable isotope ratios are reported as δ (‰) in line with the equation: $\delta_{sample} = \frac{R_{sample}}{(R_{standard})-1} \times 1000$, where R is the isotopic ratio ($^{13}C/^{12}C$ or $^{15}N/^{14}N$), and standard is an international reference value: atmospheric air for $\delta^{15}N$ and Pee Dee Belemnite (PDB) for $\delta^{13}C$. International standards were measured for calibration and measurement precision, which were < 0.1 ‰ for $\delta^{13}C$ and < 0.2 ‰ for $\delta^{15}N$. In order to facilitate comparison of the samples, we corrected the values of the historical specimens for the Suess effect (Dombrosky, 2020). The obtained values of all measurements, including the uncorrected $\delta^{13}C$ values, are available in the Supplementary Materials Table S1. We performed the analyses in the Laboratory of Biogeochemistry and Environmental Conservation at the University of Warsaw Biological and Chemical Research Centre (CNBCh UW).

2.5 Anthropogenic nitrogen input and land use characteristics

To assess the current levels of anthropogenic nitrogen input at the sample collection sites, we obtained data on the consumption of mineral nitrogen fertilisers per hectare of agricultural land within given municipalities in Poland from the 2020 General Agricultural Census (GUS, 2024). Moreover, we used European air quality data for the 2023 dataset (Anon., 2025) as a source of data on the NOx atmospheric deposition in the sampling locations. The dataset provides NOx concentrations for vegetation-related indicators (annual averages) at a 2 km² grid. It combines air quality monitoring data, chemical transport model results, and other supplementary data using a regression-interpolation-merging mapping methodology (Anon., 2025).

Using GIS tools (QGIS Development Team, 2024), we calculated the proportion of agricultural land within 10 km buffers around the contemporary specimens collection sites. We used CORINE Land Cover (CLC, 2018) as a source of land-use cover data, which has a resolution of 100 m. Within each buffer size, we calculated the sum of all subcategories of Level 1 “2. Agricultural area” occurring in our study area: 2.1.1 Non-irrigated arable land, 2.2.2 Fruit trees and berry plantations, 2.3.1 Pastures, 2.4.2 Complex cultivation patterns, and 2.4.3 Land principally occupied by agriculture, with significant areas of natural vegetation. We then divided the sum by the total area of the respective buffer.

2.6 Data analysis

We performed all the statistical analyses in R ver. 4.4.2 (R Core Team, 2024), and visualisation of the results using the 'ggplot2' R-package (Wickham, 2016). We used a two-sample, two-sided t-test with unequal variances to see whether there were differences in the mean $\delta^{15}N$ between samples of dry grassland and wetland plants in both the historical and contemporary specimen groups. Prior to other statistical tests, we calculated the differences in $\delta^{15}N$, total nitrogen content (TN) and $\delta^{13}C$ by

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subtracting the measurements obtained from historical samples from those obtained from contemporary samples belonging to the same pair. We then used 90 % confidence intervals (CIs), which equals to one sided 95 % confidence of lower or higher mean, to test whether the differences were statistically significant from zero within each of the species studied, as well as within groups of species representing the two studied habitat types (dry grassland species vs. wetland species). The calculation of the CIs was done using one sample t-test implemented in the 'ci' function from the R-package 'gmodels' (Warnes et al., 2024). To test whether differences in δ^{15}N and TN over time were influenced by the habitat type, proportion of farmland in the surrounding area, the quantity of synthetic nitrogen fertilisers used, and the amount of NOx deposited, we employed multiple linear regression. Additionally, we performed a linear regression model of δ^{13}C against δ^{15}N, incorporating all the wetland plant samples studied.

3 Results

3.1 Nitrogen isotopes and content across habitats and species

The δ^{15}N value was significantly lower in the foliar samples of dry grassland species than in wetland plant species, in both groups of contemporary and historical samples (see Fig. 2a). In contemporary samples, the difference between the means of plants representing the two habitat types was 3.83 ‰ (dry grassland species: mean δ^{15}N = -1.91 ‰; wetland species: mean δ^{15}N = 5.73 ‰), whereas in historical samples, the difference was 3.22 ‰ (dry grassland species: mean δ^{15}N = 1.24 ‰; wetland species: mean δ^{15}N = 4.45 ‰) (Fig. 2a, for more details see Appendix A Table A1 and Table A4). The value of TN was also significantly lower in the foliar samples of dry grassland species than in wetland plant species, in both contemporary and historical groups of samples (Fig. 2b). In contemporary samples, the difference between the means of TN in plants representing the two habitats was 1.06 % (dry grassland species: mean TN = 2.72 %; wetland species: mean TN = 3.78 %), whereas in historical samples the difference was 0.55 % (dry grassland species: mean TN = 2.92 %; wetland species: mean TN = 3.47 %) (Fig. 2b, Appendix A Table A2 and Table A4).

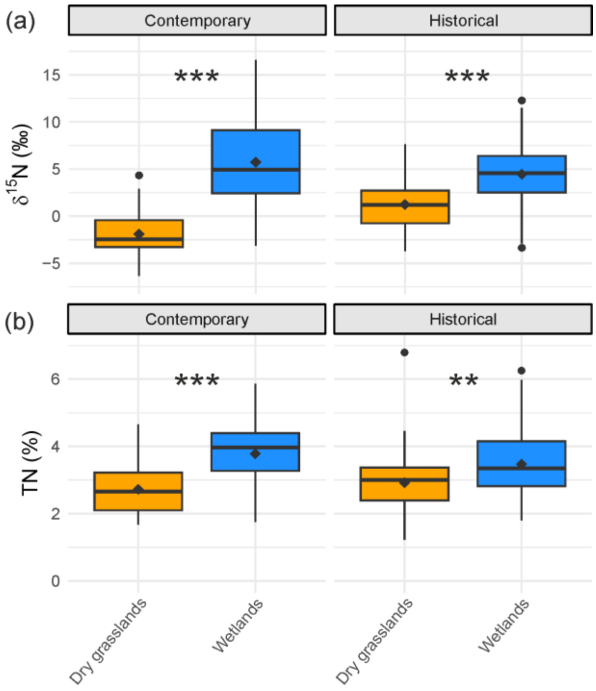


Figure 2: δ^{15}N (a) and TN (b) in historical and contemporary foliar samples from dry grassland (*n* = 51) and wetland (*n* = 49) plants. The asterisks represent the significance level based on a two-sample t-test assuming unequal variances (= $p < 0.01$, *** = $p < 0.001$). The boxes indicate the upper and lower quartiles, the black rhombus indicates the mean, and the line within a box indicates the median.**

In the dry grassland species, the mean δ^{15}N value was significantly lower in the contemporary samples than in the historical ones, with a mean difference of -3.14 ‰ (Fig. 3a). The mean difference in δ^{15}N values was lower for all species representing this habitat type, but was significant for all except one (*Carex flacca*) (Fig. 3a, Appendix A Tables A1 and A4). In the wetland species, the mean δ^{15}N value was significantly higher in the contemporary samples than in the historical ones, with a mean difference of 1.28 ‰. However, the difference was not significant in any of the single species representing this habitat (Fig. 3a, Appendix A Table A4). We did not confirm a difference in the mean TN between contemporary and historical samples of all dry grassland species pooled together (Fig. 3b). While most species in this habitat type had a lower mean TN in contemporary samples than historical ones, this difference was only significant in *Carex praecox*. Two other species showed an opposite trend, but only one of these (*Silene otites*) had a significantly higher mean TN in contemporary samples than in historical ones (Fig. 3b, Appendix A Tables A2 and A4). The mean TN was significantly higher in contemporary than in historical samples of wetland species, with a mean difference of 0.31 % (Fig. 3b, Appendix A Table A4). Significantly higher mean TN was also confirmed in three species that represent this habitat type: *Cardamine amara*, *Elodea canadensis*, and *Stuckenia pectinata* (see Fig. 3b and Appendix A Table A4). The relative mean difference of TN followed similar patterns to absolute values as shown in Appendix B Figure B1.

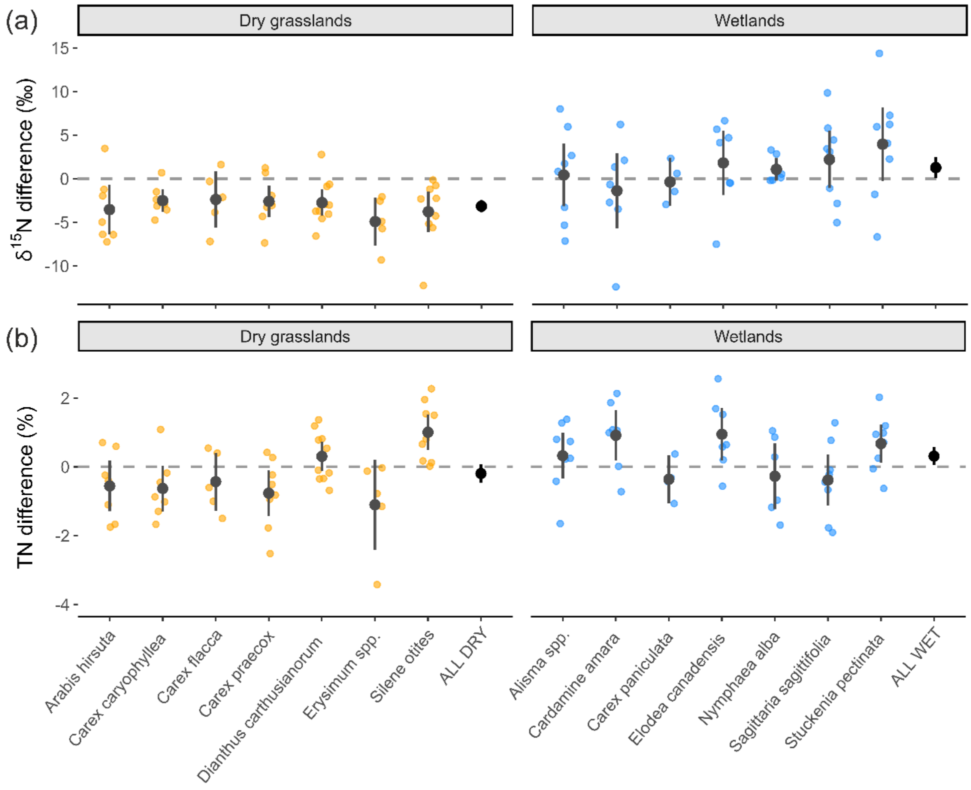


Figure 3: Mean differences between the δ^{15}N value and TN of contemporary samples and historical samples across the species and habitat types. Each observation is the difference between a contemporary and a historical sample paired with a location. The thick lines represent the 90 % confidence interval (CI) for the population mean: grey for the given species and black for all species, which represent the given habitat type.

3.2 Change of differences against nitrogen input and land cover

The difference in δ^{15}N between contemporary and historical samples paired by location was significantly higher in wetland plants than in dry grasslands and positively related to the share of farmland in a 10 km radius around the location (Table 2, for visualisation see Appendix B Figure B2). NOx deposition was also positively related with the difference in δ^{15}N, although at a lower significance level (Table 2). Among the predictors of the difference in TN between contemporary and historical samples paired by location only the habitat type was significant, indicating higher increase in TN in case of wetland plants than in dry grassland plants (Table 2).

Table 2: The results of the multiple linear regression models where the habitat type, the share of farmland within a 10 km radius, consumption of synthetic N fertilisers (average use in kg/ha per municipality), and NOx deposition were used as predictors of the difference in both δ^{15}N and TN between contemporary and historical foliar samples paired by location. Significant *p*-values ($p < 0.05$) are marked in bold.

Dependent variable	Predictors included in the model	Coeff. estimate	*p*-value	Adj.-R^2
δ^{15}N difference ~	habitat type (wetlands vs dry grasslands)	4.563	**< 0.001**	0.265
	share of farmland	7.105	**0.008**	
	synthetic N fertilisers	-0.015	0.402	
	NOx deposition	0.389	0.052	
TN difference ~	habitat type (wetlands vs dry grasslands)	0.501	**0.029**	0.016
	share of farmland	0.264	0.725	
	synthetic N fertilisers	0.003	0.623	
	NOx deposition	0.008	0.885	

3.4. Carbon isotopes across habitats and species

When samples from each habitat type were pooled, the mean δ^{13}C value, corrected for the Suess effect, was significantly lower in contemporary samples than in historical ones. The mean difference was -0.51 ‰ for dry grassland and -3.85 ‰ for wetland species (Fig. 4). A significantly lower mean difference in δ^{13}C, corrected for the Suess effect, was confirmed for *Carex praecox* among the dry grassland species and for the following five wetland species: *Alisma* spp., *Cardamine amara*, *Elodea canadensis*, *Sagittaria sagittifolia* and *Stuckenia pectinata*. The largest differences in the corrected δ^{13}C values were found between contemporary and historical samples of *E. canadensis* and *S. pectinata*, with means of -8.38 ‰ and -7.55 ‰, respectively (Fig. 4, Appendix A Table A4). We also found a negative correlation between the δ^{13}C and δ^{15}N values in these two species, but this was only significant for *S. pectinata* ($R^2 = 0.41$, $p < 0.01$; see Appendix B Figure B3).

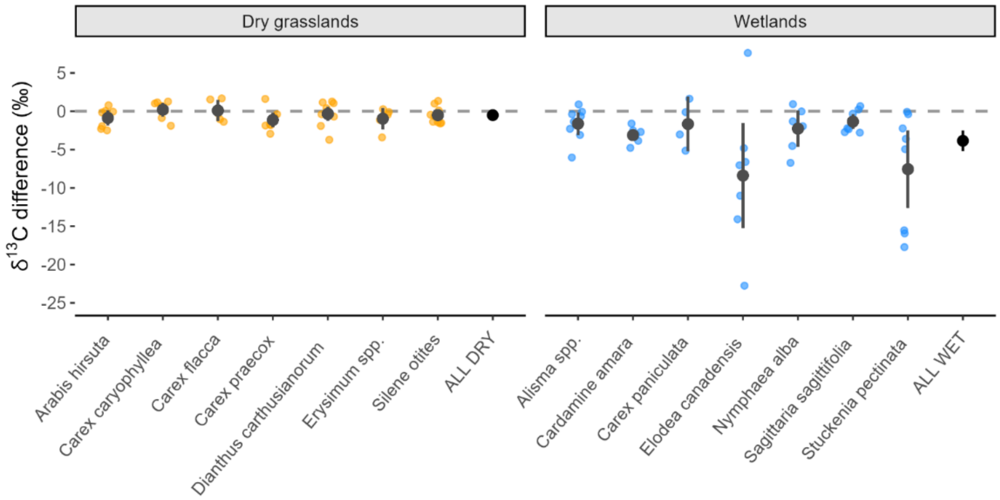


Figure 4: Mean differences between the δ^{13}C of contemporary samples and historical samples across the species and habitat types. Each observation represents the difference between contemporary and historical sample values (δ^{13}C of contemporary sample - δ^{13}C of historical sample) paired with a location. The thick lines are 90 % CI for the population mean: grey for species and black for all plants representing the given habitat type. Please note that prior to the statistical analyses the historical values were corrected for the Suess effect.

4 Discussion

The first hypothesis was confirmed for δ^{13}C, as well as δ^{15}N. In dry grassland species, we found a significant decrease in δ^{15}N values by 3.14 ‰ on average in contemporary plant leaf tissues compared to those from the pre-Green Revolution period. This is consistent with the results obtained by other authors: McLauchlan et al. (2010) detected a long-term decrease in δ^{15}N in leaves beginning in 1940 – in their study, δ^{15}N values in leaves decreased on average by 4.2‰ (change of mean value from 3.4‰ to -0.8‰) between 1940 and 2008. Similarly, Peñuelas and Filella (2001) detected a systematic decrease in mean δ^{15}N values between 1920–1930 and 1985–1995 by 3.32‰ (from 2.26‰ to -1.06‰). Peñuelas and Matamala (1990) also found a downward trend in δ^{15}N in herbarium material. These declining trends in δ^{15}N values over time have also been detected in other environmental records such as lake sediments (Holtgrieve et al., 2011), tree rings (Poulson et al., 1995; Bukata and Kyser, 2007) and ice cores (Freyer et al., 1996; Hastings et al., 2009). McLauchlan et al. (2010) related the decreasing trend in foliar δ^{15}N of plants from the North American prairie with increasing concentration of CO_2 in the atmosphere, leading to stronger N-limitation of primary productivity. The decrease in nitrogen availability is usually associated with a stronger decrease in δ^{15}N values in leaves (Caldararu et al., 2022). However, when we compared changes in the total concentration of nitrogen in leaf tissues, our study did not confirm the trend of increasing nitrogen limitation resulting from the carbon fertilisation of ecosystems observed by McLauchlan et al. (2010). The decrease in the δ^{15}N values of dry grassland plants observed in our study can be explained by several mechanisms, which are not mutually exclusive. Firstly, the cessation of free-range grazing and area of pastures in Poland over the last few decades (Radkowska and Radkowski, 2023; Sucholas et al., 2025) has caused livestock and their ^{15}N-enriched faeces (Bateman and Kelly, 2007) to disappear from dry grasslands. In fact, the vast majority of our samples were collected from dry grasslands that, in recent years, were only mown or were undergoing secondary succession after abandonment of grazing. Secondly, and probably the most important driver, is the atmospheric deposition that has become an important source of nitrogen depleted in ^{15}N (Hastings et al., 2009). Such reasoning was also proposed by Peñuelas and Filella (2001) to explain the declining trend in foliar δ^{15}N of plants from dry habitats in Spain. They suggested that in dry conditions, the nitrogen pool is more dependent on atmospheric deposition than in the case of more humid and cold conditions, which promote microbial activity and organic nitrogen mineralisation. Our results may indicate that these vulnerable habitats may be receiving a particularly high input of anthropogenic nitrogen via atmospheric deposition, which can reach areas that are remote from towns and farms (Holtgrieve et al., 2011).

In contrast, the δ15N values of wetland species had increased slightly compared to the pre-Green Revolution period. While confirming the first hypothesis, this also supports our second hypothesis. Such results could be explained by the dominance of nitrogen pollution derived from organic matter (with higher δ15N values) in aquatic habitats. This is due to human sewage entering surface water and groundwater (Bateman and Kelly, 2007), which can sometimes result in extremely high δ15N levels (Alldred et al., 2024). Despite significant progress in wastewater treatment in Poland in recent decades, environmental monitoring of rivers and lakes shows high levels of organic waste contamination (Szołdrowska and Smol, 2025). Our δ13C results for wetland plants (discussed below) support this explanation, as they also suggest that increased water pollution by allochtonous organic matter is probably the main driving force behind the observed changes in wetlands. In comparison with the pre-Green Revolution era, there is a possibility that the level of organic (nitrogen and carbon) pollution may be rising due to factors other than the quantity of organic fertilisers. Such factors may include the method and distribution of manure fertilisation (which was less intensive and more scattered in the past than in modern agriculture), uncontrolled sewage inputs from intensively developing settlements and tourist infrastructure close to rivers and lakes, and the runoff of peat from ploughed organic soils or drained peatlands (a fairly common phenomenon in Poland) (Krüger et al., 2015; Heredia et al., 2022). Alternatively, the observed pattern in wetland species could be attributed to increased runoff of artificial nitrogen fertilisers into water systems, leading to elevated nitrogen concentrations and resulting in higher rates of organic nitrogen mineralisation in water, followed by increased rates of nitrification, leading to elevated δ^{15}N values (Diebel and Zanden, 2009). This is particularly relevant given that the use of organic fertilisers in European agriculture has remained at similar levels to those observed before the Green Revolution (Tian et al., 2022). In dry habitats, the complex microbial processes leading to 15N enrichment, as previously described for wetlands, are hampered due to water deficits; thus, the inorganic nitrogen resulting from atmospheric deposition (15N-depleted) may serve more directly as a source of that macroelement for plants.

It is worth noting that there was variation in the δ^{15}N difference among the wetland species studied. The increase in δ^{15}N was particularly high in the submerged species *Stuckenia pectinata*, which would agree with our second hypothesis, but the temporal change for this species was not statistically significant. Generally, the variation in δ^{15}N difference within this group was greater than in dry grassland species, making statistical inference more challenging (given the relatively small number of observations). Overall, changes to wetland habitats in Poland over the last century seem to be relatively significant not only due to ongoing pollution, but also due to widespread drainage of wetlands for agricultural purposes (Janssen et al., 2016), increasingly frequent droughts leading to increased concentrations of pollutants (Szołdrowska and Smol, 2025) and rise of average water temperature following the climate change and other anthropogenic impacts (Tomalski et al., 2025). Thus, in many cases, finding current specimens of wetland species in the locations indicated in the herbaria was challenging or even impossible due to the disappearance of wetlands, profound changes in their trophic state, or changes to the surrounding landscape. Consequently, the sources of nitrogen and their isotopic signature may evolve in wetlands more complexly over time than in dry grasslands, making it difficult to observe general trends in this group.

We found that the change in the total nitrogen content of the foliar samples varied greatly depending on the species and habitat. The increase predicted by our third hypothesis was confirmed only for wetland species. In the case of dry grassland species we observed no general trend of TN changes over time. At the species level one species, *Carex praecox*, showed statistically significant decline as observed by McLauchlan et al. (2010) who attributed TN decrease observed in prairie plants in Kansas to CO_2 fertilisation effects and increase of water use-efficiency under elevated CO_2 (especially important in dry habitats where primary productivity could be limited by drought) which could lead to increase in primary productivity and finally to productivity limitation by nitrogen. On the other hand, in our study, *Silene otites* showed the significantly opposite pattern, with an increase in TN, despite usually occupying habitats with extreme water deficits. *Dianthus carthusianorum* also had a slightly increased TN (although not significant), which may suggest a family-specific evolutionary strategy, as both species belong to the *Caryophyllaceae* family. On the other hand we also did not observe a general increase in foliar nitrogen content as Kühn et al. (2025). This may mean that for most of the species additional input of anthropogenic nitrogen have compensated for the increased demand for this nutrient related to the rising carbon dioxide levels but has not exceeded it as happened in probably even more overfertilised habitats studied by Kühn et al. (2025).

In the case of wetland plants there was an overall visible increase of total nitrogen content in plant tissues when contemporary plants were compared with herbaria vouchers from the pre-Green Revolution period. This trend was driven by strong and statistically significant responses in *Cardamine amara*, *Elodea canadensis*, and *Stuckenia pectinata* where we observed relative increases in tissue total nitrogen concentrations of more than 20 % (see Appendix B Figure B1). Those species were not standing out in the case of $\delta^{15}N$ difference, but their $\delta^{13}C$ difference was significantly bigger than expected from the global ambient $\delta^{13}C$ trends. Both could point to water bodies being polluted by sewage and organic fertilisers, bringing both ^{15}N-enriched nitrogen and ^{13}C-enriched organic carbon, which, after mineralisation, is turned into DIC, which is utilised by those, mostly submerged, plants.

We confirmed our fourth hypothesis only for the trends in $\delta^{15}N$. The decline of $\delta^{15}N$ in foliar samples was larger in areas with a lower share of farmland in the surrounding landscape. There are a number of studies that found positive correlations between the anthropogenic nitrogen load and $\delta^{15}N$ values in diverse elements of the environment. For example, Xue et al. (2009) showed a significant positive relationship between river NO3- $\delta^{15}N$ values and the proportion of agricultural and urban land use. Dailer et al. (2010) obtained low $\delta^{15}N$ values in algae collected from areas with low anthropogenic influence. An increase in $\delta^{15}N$ related to agricultural and urban areas was found in macrophytes from coastal (Kohzu et al. 2008) and river ecosystems (Pronin et al., 2025). Alternatively, our results can be attributed to the increased use of fertiliser in agriculture today compared to the past (Tian et al., 2022), regardless of whether they are organic or artificial. Intensively used agricultural land, as a result of the use of nitrogen fertilisers, manure and other agricultural practices, is characterised by increased nitrogen availability and a higher rate of nitrogen circulation, resulting in greater losses of this element through processes such as denitrification, ammonia volatilisation and nitrate leaching (Craine et al., 2009; Hall et al., 2015; Gerschlauer et al., 2019). As a result, the remaining nitrogen pool is gradually enriched with ^{15}N, which may be reflected in high δ^{15}N values in leaves (Gubsch et al., 2011).

We did not fully confirm our fifth hypothesis. After applying the correction for the Suess effect, we were left with a small (0.51 ‰) but significant decrease in δ^{13}C since historical times in dry grassland plant tissues. This is contrary to the expected increase in water-use efficiency (Seibt et al. 2008) caused by water shortage, and is hard to explain. The significant decrease in δ^{13}C in wetland plants agrees with our second hypothesis. Wetland plants can show such a response to eutrophication due to two mechanisms: 1) increased nutrient availability causes higher productivity that causes plants to increase stomatal conductance (in not fully submerged plants), which leads to higher fractionation (Wang et al. 2015). The parameter strongly correlated with δ^{13}C in most studies with such results (Chappuis et al. 2017, Wrosz et al. 2025) is total phosphorus in water. We do not have such values for our study, but we can safely assume that P has increased due to anthropogenic input. 2) An oversupply of organic matter (with “terrestrial” δ^{13}C values) from sewage and organic fertiliser into wetlands causes intensive bacterial mineralisation. At some point, most DIC can originate from this process, thus has very low δ^{13}C values (Hellings et al. 1999). Heredia et al. (2022) attributed the decrease in δ^{13}C of submerged plants in an eutrophication gradient to allochtonous organic matter decomposition. Similar findings - surprisingly low δ^{13}C values - came from the study on urban rivers in NW Poland (Pronin et al. 2025). The simultaneous response on δ^{13}C and δ^{15}N (Appendix B Figure B2) confirms that eutrophication is the main driver for these changes and an important factor affecting wetland ecosystems at a large scale.

5 Conclusions

The present study revealed that, whilst the isotopic composition of nitrogen and carbon in plant tissues in Central Europe has changed over the last century, this change is very context-dependent. The magnitude and direction of the observed change are impacted by both the habitat type and the species identity and/or ecology. This may be regarded as a caveat for future researchers, suggesting that both the taxonomy and ecology of stable isotopes in plant tissues should be considered in studies. Nevertheless, the anthropogenic global change has left an imprint on the isotopic composition of the flora from the habitats under study. As would be expected, the alterations appear to be more pronounced in wetland environments than in dryland habitats. It is evident that both habitat types are among the most threatened in Poland (Perzanowska and Korzeniak, 2022) and the entire European Union (Janssen et al., 2016). However, it appears that human activity has resulted in more significant alterations to biogeochemical processes in wetlands compared to dry grasslands. While the latter have also diminished in size, the functionality of the remnants appears to be less affected. Moreover, the source of this disruption may vary between the two habitat types. Specifically, wetlands are exposed to a multitude of anthropogenic nitrogen and carbon sources, while dry grasslands are predominantly affected by alterations in atmospheric composition.

Data availability

All the data used in the study are available in the supplementary files.

Appendix A

Table A1: Summary statistics of δ^{15}N (values given in ‰) of the foliar samples of 14 species selected for the study, from both contemporary specimens collected in 2024 and historical specimens in the form of herbarium vouchers collected before 1939.

Taxon/group	Type of specimen	*n*	Min.	Max.	Median	Mean	SD	SE
Alisma spp.	contemporary	8	-3.13	12.77	5.45	5.06	5.20	1.84
	historical	8	2.48	7.77	4.17	4.62	1.74	0.62
Arabis hirsuta	contemporary	7	-3.31	4.33	-2.47	-1.48	2.72	1.03
	historical	7	-2.01	5.39	1.72	2.04	2.53	0.96
Cardamine amara	contemporary	7	-0.10	10.50	3.60	4.32	3.36	1.27
	historical	7	0.63	12.28	4.27	5.68	3.88	1.47
Carex caryophyllea	contemporary	7	-6.35	-2.41	-3.06	-3.67	1.46	0.55
	historical	7	-3.19	1.15	-1.36	-1.18	1.36	0.51
Carex flacca	contemporary	5	-3.95	2.90	0.99	-0.20	3.12	1.39
	historical	5	-0.11	4.10	2.43	2.16	1.63	0.73
Carex paniculata	contemporary	4	0.74	8.54	3.11	3.87	3.31	1.66
	historical	4	0.12	11.48	2.66	4.23	5.17	2.59
Carex praecox	contemporary	8	-3.26	1.61	-2.21	-1.26	1.93	0.68
	historical	8	-0.80	5.36	0.78	1.32	1.94	0.69
Dianthus carthusianorum	contemporary	10	-5.92	-0.59	-2.91	-3.03	1.64	0.52
	historical	10	-3.72	3.10	-1.24	-0.31	2.23	0.71
Elodea canadensis	contemporary	7	-2.40	11.17	4.39	4.89	4.52	1.71
	historical	7	-3.36	6.47	4.85	3.07	3.71	1.40
Erysimum spp.	contemporary	5	-4.09	0.41	-1.21	-1.53	2.05	0.92
	historical	5	0.80	6.16	2.94	3.37	2.04	0.91
Nymphaea alba	contemporary	6	-0.22	8.50	2.31	3.24	3.57	1.46
	historical	6	-2.90	6.49	2.47	2.16	3.53	1.44

Sagittaria sagittifolia	contemporary	8	1.49	16.28	8.52	8.01	4.83	1.71
	historical	8	4.31	9.43	5.35	5.79	1.72	0.61
Silene otites	contemporary	9	-4.61	1.37	-1.32	-1.34	2.15	0.72
	historical	9	-1.32	7.64	2.13	2.43	2.49	0.83
Stuckenia pectinata	contemporary	8	-0.29	16.56	8.93	8.89	5.79	2.05
	historical	8	-1.09	9.58	5.60	4.92	3.56	1.26
All dry grassland species	contemporary	51	-6.35	4.33	-2.47	-1.91	2.27	0.32
	historical	51	-3.72	7.64	1.20	1.24	2.48	0.35
All wetland species	contemporary	48	-3.13	16.56	4.93	5.73	4.77	0.69
	historical	48	-3.36	12.28	4.55	4.45	3.32	0.48

Table A2: Summary statistics of total nitrogen content (TN, values given in %) in the foliar samples of 14 species selected for the study from both contemporary specimens collected in 2024 and historical specimens in the form of herbarium vouchers collected before 1939.

Taxon/group	Type of specimen	*n*	Min.	Max.	Median	Mean	SD	SE
Alisma spp.	contemporary	8	3.06	4.49	3.98	3.77	0.52	0.18
	historical	8	1.88	4.96	3.45	3.44	1.00	0.35
Arabis hirsuta	contemporary	7	1.93	3.33	2.73	2.70	0.56	0.21
	historical	7	2.34	4.41	3.44	3.25	0.80	0.30
Cardamine amara	contemporary	7	3.44	5.86	4.51	4.51	0.86	0.32
	historical	7	2.39	4.51	3.73	3.59	0.86	0.33
Carex caryophyllea	contemporary	7	1.91	2.69	2.21	2.19	0.28	0.11
	historical	7	1.26	3.59	3.08	2.81	0.77	0.29
Carex flacca	contemporary	5	1.87	2.23	2.01	2.01	0.15	0.07
	historical	5	1.47	3.51	2.47	2.44	0.94	0.42
Carex paniculata	contemporary	4	1.75	2.77	2.36	2.31	0.42	0.21
	historical	4	2.06	3.20	2.71	2.67	0.48	0.24
Carex praecox	contemporary	8	1.67	3.27	2.16	2.37	0.58	0.21

	historical	8	2.07	4.19	3.03	3.13	0.66	0.23
Dianthus carthusianorum	contemporary	10	1.92	3.73	2.52	2.61	0.53	0.17
	historical	10	1.22	3.15	2.40	2.31	0.66	0.21
Elodea canadensis	contemporary	7	3.45	4.88	4.50	4.30	0.57	0.21
	historical	7	1.94	4.49	3.36	3.35	0.85	0.32
Erysimum spp.	contemporary	5	2.28	3.37	3.28	3.02	0.47	0.21
	historical	5	3.31	6.79	3.50	4.13	1.49	0.67
Nymphaea alba	contemporary	6	1.84	4.10	3.03	2.98	0.83	0.34
	historical	6	2.52	3.86	3.27	3.25	0.45	0.19
Sagittaria sagittifolia	contemporary	8	3.94	5.37	4.27	4.35	0.45	0.16
	historical	8	3.42	6.25	4.55	4.73	0.94	0.33
Silene otites	contemporary	9	3.14	4.65	4.01	3.82	0.61	0.20
	historical	9	1.64	4.45	2.70	2.82	0.80	0.27
Stuckenia pectinata	contemporary	8	2.10	4.59	3.74	3.47	0.99	0.35
	historical	8	1.80	3.65	2.92	2.80	0.69	0.24
All dry grassland species	contemporary	51	1.67	4.65	2.66	2.72	0.75	0.11
	historical	51	1.22	6.79	3.00	2.92	0.95	0.13
All wetland species	contemporary	48	1.75	5.86	3.97	3.78	0.94	0.14
	historical	48	1.80	6.25	3.35	3.47	0.99	0.14

420 **Table A3: Summary statistics of δ^{13}C (values given in ‰) of the foliar samples of 14 species selected for the study, from both contemporary specimens collected in 2024 and historical specimens in the form of herbarium vouchers collected before 1939. Please note that the historical values were corrected for the Suess effect.**

Taxon/group	**Type of specimen**	***n***	**Min.**	**Max.**	**Median**	**Mean**	**SD**	**SE**
Alisma spp.	contemporary	8	-35.26	-28.42	-30.60	-30.86	2.26	0.80
	historical	8	-29.88	-28.36	-29.29	-29.24	0.56	0.20
Arabis hirsuta	contemporary	7	-33.00	-29.81	-30.98	-31.26	1.06	0.40
	historical	7	-31.37	-28.19	-30.81	-30.39	1.10	0.42

Cardamine amara	contemporary	7	-34.24	-30.59	-33.04	-33.02	1.31	0.49
	historical	7	-31.41	-28.99	-29.72	-29.91	0.83	0.31
Carex caryophyllea	contemporary	7	-30.58	-28.68	-29.51	-29.50	0.61	0.23
	historical	7	-31.28	-27.98	-30.18	-29.71	1.24	0.47
Carex flacca	contemporary	5	-31.55	-28.06	-28.99	-29.58	1.46	0.66
	historical	5	-30.34	-27.95	-30.18	-29.68	1.01	0.45
Carex paniculata	contemporary	4	-33.67	-26.70	-29.29	-29.74	3.06	1.53
	historical	4	-28.53	-27.49	-28.14	-28.08	0.46	0.23
Carex praecox	contemporary	8	-30.84	-28.52	-29.74	-29.65	0.73	0.26
	historical	8	-30.13	-26.84	-28.55	-28.52	1.01	0.36
Dianthus carthusianorum	contemporary	10	-30.65	-28.47	-29.50	-29.49	0.70	0.22
	historical	10	-31.21	-26.50	-29.10	-29.15	1.29	0.41
Elodea canadensis	contemporary	7	-36.72	-22.75	-31.66	-30.95	4.65	1.76
	historical	7	-39.27	-13.97	-21.04	-22.56	8.07	3.05
Erysimum spp.	contemporary	5	-29.82	-27.81	-29.29	-29.05	0.76	0.34
	historical	5	-29.73	-25.94	-28.16	-28.08	1.37	0.61
Nymphaea alba	contemporary	6	-31.51	-24.53	-27.01	-27.23	2.64	1.08
	historical	6	-26.56	-23.99	-24.78	-24.96	0.94	0.38
Sagittaria sagittifolia	contemporary	8	-31.45	-28.11	-29.08	-29.68	1.35	0.48
	historical	8	-32.12	-25.78	-28.39	-28.34	1.88	0.66
Silene otites	contemporary	9	-30.36	-27.27	-29.01	-28.84	1.01	0.34
	historical	9	-29.24	-27.49	-28.62	-28.34	0.60	0.20
Stuckenia pectinata	contemporary	8	-42.17	-14.33	-29.72	-29.31	8.56	3.03
	historical	8	-30.02	-13.95	-22.39	-21.76	5.21	1.84
All dry grassland species	contemporary	51	-33.00	-27.27	-29.54	-29.61	1.12	0.16
	historical	51	-31.37	-25.94	-28.80	-29.10	1.28	0.18
All wetland species	contemporary	48	-42.17	-14.33	-30.72	-30.19	4.35	0.63

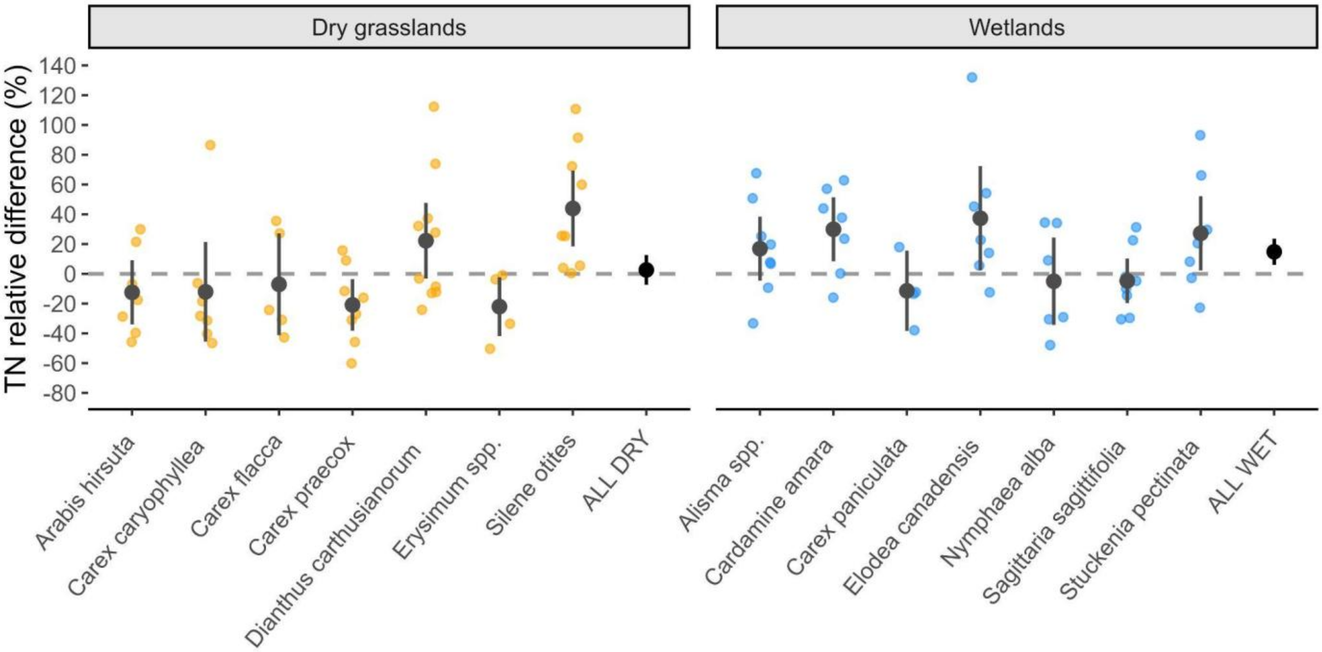
	historical	48	-39.27	-13.95	-28.00	-26.34	4.83	0.70

Table A4: Mean differences between the δ^{15}N, TN, and δ^{13}C of contemporary samples and historical samples across the species and habitat types. The lower and upper 90 % confidence intervals for means were calculated using a one-sample t-test.

		δ^{15}N difference (‰)			TN difference (%)			δ^{13}C difference (‰)		
Taxon/group	***n***	**mean**	**lower CI**	**upper CI**	**mean**	**lower CI**	**upper CI**	**mean**	**lower CI**	**upper CI**
Alisma spp.	8	0.44	-3.13	4.01	0.32	-0.34	0.99	-1.62	-3.08	-0.16
Arabis hirsuta	7	-3.52	-6.35	-0.69	-0.55	-1.29	0.18	-0.87	-1.85	0.10
Cardamine amara	7	-1.36	-5.66	2.94	0.91	0.19	1.64	-3.11	-3.86	-2.37
Carex caryophyllea	7	-2.49	-3.76	-1.22	-0.63	-1.29	0.04	0.20	-0.67	1.08
Carex flacca	5	-2.36	-5.58	0.85	-0.43	-1.28	0.41	0.10	-1.26	1.46
Carex paniculata	4	-0.36	-3.09	2.38	-0.36	-1.06	0.33	-1.66	-5.21	1.89
Carex praecox	8	-2.58	-4.42	-0.74	-0.77	-1.43	-0.10	-1.13	-2.04	-0.22
Dianthus carthusianorum	10	-2.73	-4.22	-1.23	0.30	-0.11	0.72	-0.33	-1.23	0.56
Elodea canadensis	7	1.82	-1.84	5.49	0.95	0.18	1.71	-8.38	-15.22	-1.55
Erysimum spp.	5	-4.90	-7.67	-2.13	-1.10	-2.41	0.21	-0.96	-2.36	0.43
Nymphaea alba	6	1.08	-0.21	2.38	-0.27	-1.22	0.68	-2.27	-4.63	0.10
Sagittaria sagittifolia	8	2.22	-1.06	5.49	-0.38	-1.12	0.36	-1.34	-2.26	-0.41
Silene otites	9	-3.77	-6.07	-1.46	1.00	0.48	1.52	-0.51	-1.20	0.18
Stuckenia pectinata	8	3.97	-0.25	8.19	0.68	0.13	1.22	-7.55	-12.59	-2.52
All dry grassland species	51	-3.14	-3.84	-2.44	-0.20	-0.46	0.07	-0.51	-0.83	-0.19
All wetland species	48	1.28	0.08	2.48	0.31	0.05	0.57	-3.85	-5.18	-2.52

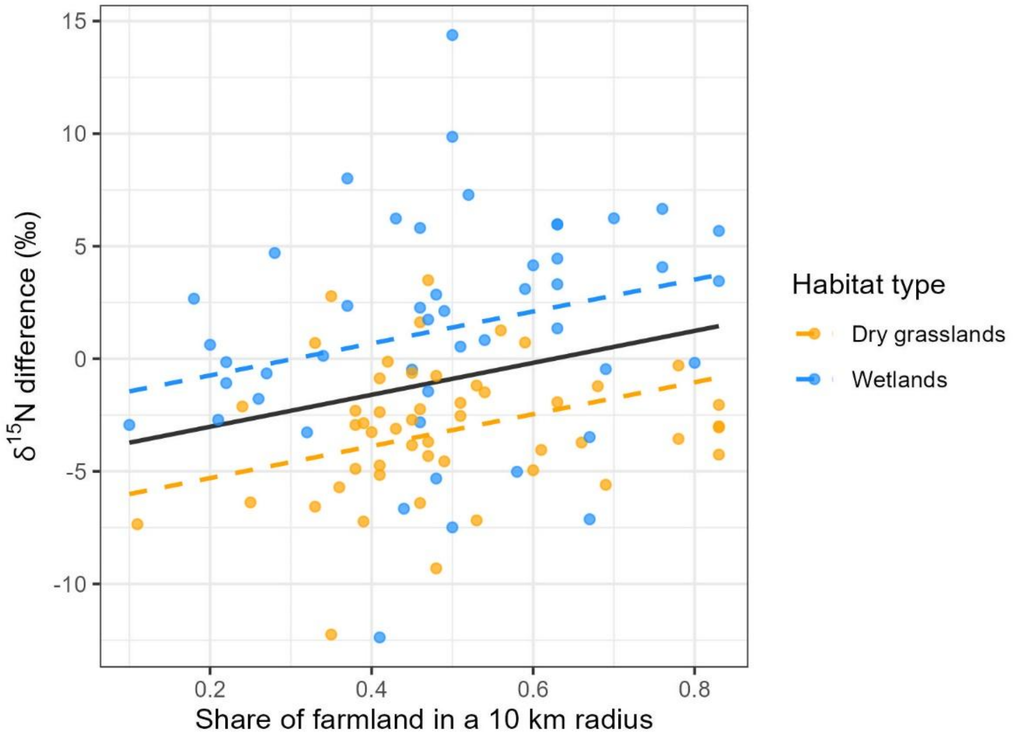
Appendix B

https://doi.org/10.5194/egusphere-2025-5959
Preprint. Discussion started: 5 January 2026



430 **Figure B1: Relative mean differences between the TN of contemporary samples and historical samples across the species and habitat types. Each observation is the difference between a contemporary and a historical sample paired with a location. The relative difference was calculated according to a formula: (TN contemporary sample - TN historical sample)/TN historical sample*100. The thick lines represent the 90 % confidence interval (CI) for the population mean: grey for the given species and black for all species, which represent the given habitat type.**

435



440 **Figure B2: The relationship between the proportion of farmland within a 10 km radius and the difference in δ^{15}N between contemporary and historical foliar samples, which were paired by location. The black line represents the predicted value of the dependent variable for each value of the main independent variable, while accounting for the influence of the other independent variables. The dashed lines represent the regression lines for the given habitat type.**

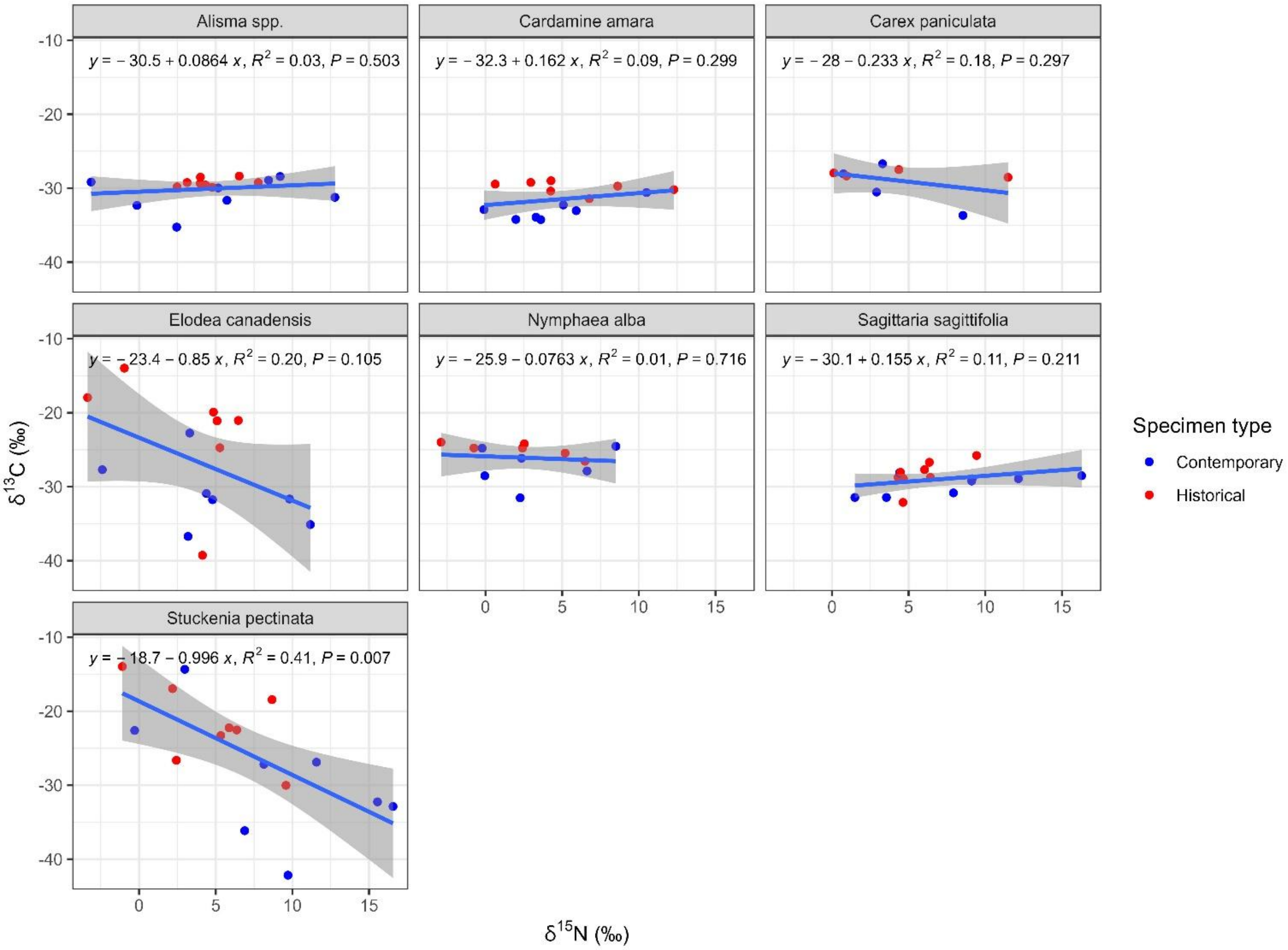


Figure B3: The relationships between δ^{15}N and δ^{13}C in all the foliar samples studied (i.e. historical and contemporary samples combined) from seven wetland species. The blue line shows the fitted linear regression model, and the grey area shows the 95 % confidence interval.

Supplementary Materials

Table S1: List of all foliar samples and the raw data used in the analyses.

Author contributions

ID and ŁK conceived the idea, designed, and supervised the project. ID, ŁK and NC conducted field sampling. NC and ID selected and sampled the herbarium material. PC performed the laboratory analyses. NC obtained the GIS data and prepared the final dataset. ID analysed the data and prepared the figures, besides the map, which was prepared by ŁK. Writing was led by ID, with support from all other authors.

Competing interests

The authors declare that they have no conflict of interest.

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