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RESEARCH ARTICLE

Nitrogen content of herbarium specimens from arable fields and mesic meadows reflect the intensifying agricultural management during the 20th century

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Abstract

1. Arable fields and mesic meadows have been affected by intensifying agricultural management and nutrient input during the 20th century, but direct evidence for the long-term impact of intensification on plant nutrient contents remains scarce. Non-destructive novel spectroscopic methods can produce such data from herbarium specimens, making it possible to investigate how contents of leaf nutrient traits, especially nitrogen and phosphorus, changed over the last century, and what role habitat type and management practices play.
2. We carried out a resurvey study of functional traits in arable field and mesic meadow communities. We used specimens from two German herbaria with a high coverage of their local floras: the herbaria Senckenberg Görlitz and Senckenberg Haussknecht in Jena. Following specimen information, the same plant species were resampled in the field in 2022 at the same locations. We employed near-infrared spectroscopy to predict leaf nitrogen, phosphorus and carbon content of herbarium and field specimens. Nutrient content changes over time were compared with public records of regional P and N fertilization.
3. Overall, 1270 specimens of 76 species from both herbarium and field were studied, the oldest from the 19th century. Leaf nitrogen and the leaf nitrogen:phosphorus ratio increased significantly through time, while leaf phosphorus and carbon content decreased significantly over time. Arable field species showed a stronger response in leaf phosphorus content and leaf nitrogen:phosphorus ratio than mesic meadow species. The total amount of nitrogen or phosphorus fertilizer applied per year on a regional scale was found to be significantly correlated with the respective leaf nutrient content levels.

Paul Kühn and Raymond Umazekabiri contributed equally to this study and should be considered joint first authors.

4. *Synthesis*: Our study shows a long-term increase of leaf nitrogen in the studied habitats, paralleling increased chemical fertilizer applications in Germany. Our data indicate a shift from predominantly N-limited towards more P-limited growth conditions. The stronger response of species from arable fields compared to species from mesic meadows could indicate a faster adjustment to environmental pressures. This study thus also serves to showcase the potential of the combination of herbarium collections and NIR spectroscopy.

KEYWORDS
biological collection, eutrophication, global change ecology, leaf phosphorus content, near-infrared spectroscopy, plant functional traits, resurvey

1 | INTRODUCTION

Changes in biological diversity are a key challenge for science and society and one of the most pressing environmental issues of our time (Pereira et al., 2012). Over the past 150 years, dramatic change has been detected, and anthropogenic activities rank as top drivers of biodiversity changes and losses (Chapin et al., 2000; Jaureguiberry et al., 2022). The rapid intensification and industrialization of agriculture worldwide, especially in the second half of the 20th century, has severely affected the composition and functioning of arable fields and grasslands in increasingly industrialized agricultural systems.

These two habitat types are of interest because they cover the most important agricultural areas in Europe: in Germany, around 50% of the land area is dedicated to agriculture, and of that, 70% are arable fields and 28% grasslands (BMEL, 2022). Arable fields are used for crops such as wheat, maize or oil seed grown as monocultures which are sown, grown and harvested within the span of a year while being intensively managed. This creates a habitat with very specialized conditions for plant growth and propagation favouring fast-growing annual plant species (Storkey et al., 2012). Mesic meadows are used to produce biomass as livestock fodder, silage or (more recently) energy crops, with the harvesting frequency ranging from once to five times a year. These grasslands can be species-rich, but support increasing proportions of few fast-growing and highly regenerative grass species as management is intensified (Wesche et al., 2012). Despite these differences, both habitats suffered severe losses in species richness and abundance (Meyer et al., 2013; Wesche et al., 2012), which is critical because they cover a large amount of land and harbour a large fraction of regional biodiversity. The most important drivers of these declines are intensified agricultural management practices including pesticide application, increased mowing as well as ploughing activities and mineral fertilizer inputs (Duprè et al., 2010; Robinson & Sutherland, 2002; Spahillari et al., 1999).

Functional traits in general and leaf traits in particular represent a means to gain insights into how plant species allocate resources, react to different environmental pressures and adjust growth strategies. As such, they can reflect a shift towards faster-growing, acquisitive growth strategies (Reich, 2014), which can occur when a plant community starts trending towards competitive, nutrient-demanding plants (Wesche et al., 2012). There is evidence for a variety of different trends in functional traits, which have been induced by ongoing environmental changes, including a decline of stress-resistant species (Smart et al., 2005), or a comparative decrease of leaf nitrogen values due to an increase in atmospheric CO_2 and a corresponding increase in the percentage of leaf carbon (McLauchlan et al., 2010). Such shifts in growth strategies and community structure are enhanced by fertilization regimes, which intensified over the course of the 20th century (Baessler & Klotz, 2006).

Nitrogen and phosphorus are key plant leaf nutrients (Ordoñez et al., 2009) and consequentially influence other leaf traits such as the specific leaf area, being associated with shifts to values typical of acquisitive growth strategies (Fan et al., 2022). Many ecosystems are nitrogen limited (LeBauer & Treseder, 2008), and legumes and a few other specialized plants can fix nitrogen in symbiosis with rhizobium bacteria and make it available to the local plant community (Adams et al., 2016). In industrial agriculture, massive nitrogen fertilization can lead to a switch from nitrogen-limited to phosphorus-limited growing conditions as indicated by an increasing N:P ratio (Güsewell, 2004). This is in line with the notion that N input from fertilization and atmospheric deposition remains high, while P fertilization is declining in response to rising costs and atmospheric P deposition typically is low (Güsewell, 2004). A recent study has found that increased nitrogen fertilization in general will result in reduced phosphorus availability in the soil over time (Chen et al., 2021), exacerbating the trend. Furthermore, increased soil nutrient availability has been found to primarily and negatively impact the abundance of specialized, small-ranged species, thus establishing a connection to the wider topic of biological conservation (Römermann et al., 2008; Staude et al., 2020). Given that classical monitoring and related studies of plant communities and traits rarely span more than a few decades, evidence on long-term trends in nutrient limitation is, however, limited.

Management methods of arable fields and mesic meadows are similar with respect to a focus on the production of a yearly harvest but differ in the environmental filters they impose on plant species. In arable fields, agricultural weeds have adapted to the annual cycle of the growth and harvest of the crop (Deike et al., 2008; Steinmann & Dobers, 2013). Agricultural weeds can serve as indicator species for agricultural intensification, as they face pressure through improved seed cleaning techniques, herbicide use and higher competition due to fertilization in conjunction with optimized crop breeds, which grow and absorb nutrients faster (Meyer et al., 2015; Nieder & Richter, 2000; Wietzke et al., 2020). In contrast, mesic meadows are dominated by perennial species and can be very species-rich while still being productive. However, more frequent mowing and increased fertilization has led to a decline in diversity, with an increasing number of mesic meadows now dominated by just a few tall grass species (Wesche et al., 2012).

Nitrogen and phosphorus fertilization has been observed to lead to denser, faster-growing crops (Chen et al., 2021). The amount of nitrogen fertilizer applied per hectare and year can by itself be used as a metric for the intensity of agricultural management (Baessler & Klotz 2006; Kleijn et al., 2008). In the context of Germany, the government provides tables which state the 'nutrient demand' for intensive crop production. These constitute upper limits for the total amount of nitrogen present in the soil needed for optimal, high-yield growth, adding up nitrogen sourced from chemical weathering, nitrogen fixed by previously planted crops and fertilization input. These tables state an overall requirement of up to 200 N ha kg^{-1} a^{-1} for rapeseed and maize, and between 210 and 260 N kg ha^{-1} a^{-1} for wheat. Intensive grasslands are stated to require a comparable 205 N kg ha a, while the requirement of extensively managed grasslands is as low as 73 N kg ha^{-1} a^{-1} (LfL, 2023). Some long-term experiments have investigated trends in community composition brought about by these changes (Silvertown et al., 2006). While these operate on the scale of decades, investigations of trait changes on longer time scales best rely on analysis of specimens maintained in herbarium collections, which can date back several centuries (Jaroszynska et al., 2023). Over time, plants have been collected and deposited in herbaria for various purposes. It is estimated that 338 million specimens are stored in around 3100 herbaria worldwide, with >23 million specimens accommodated in Germany's 70 herbaria alone, which ranks third place in Europe with respect to the total size (Borsch et al., 2020; Thiers, (updated continuously n.d.). The collections cover a wide range of spatial and temporal scales, from past collections abroad to extensive samplings of recent local floras, in some cases providing a temporal spread of multiple samplings from one location.

Herbaria are therefore among the largest archives of biological material and are increasingly being employed for a wide range of approaches. Studies of biogeography and phenology have benefited from the use of herbarium specimens (Greve et al., 2016; Primack et al., 2004). More recent papers (Lang et al., 2019; Meineke et al., 2018) have highlighted efforts to use herbaria for the analysis of long-term trends, while most other studies rely on fresh material and cover hardly more than the last few decades (Koschorreck et al., 2023). Herbarium specimens have been used directly in an earlier study to assess changes in tissue contents of nitrogen, and to a much lesser extent of phosphorus and other macro-nutrients, and revealed that leaf N content decreased in the second half of the 20th century as an effect of increased atmospheric CO_2 concentrations, greater carbon availability and a corresponding carbon uptake in the leaf (Peñuelas & Filella, 2001). In addition, assessing temporal changes in grassland ecosystem dynamics by using herbarium records, McLauchlan et al. (2010) found evidence for decreasing leaf nitrogen content levels over time due to an increasing abundance and availability of carbon derived from atmospheric CO_2. However, the destructive leaf nutrient analysis methods employed by the two previously mentioned studies are to be avoided when it comes to historic herbarium specimens, since it leaves irreplaceable specimens permanently diminished. Furthermore, it makes repeated studies of these samples, or studies of different traits, less feasible, as more and more material would have to be removed.

Recently, near-infrared reflectance spectroscopy (NIRS), a non-destructive approach, has been used to extract morphological and chemical leaf trait data in a non-destructive manner, for example to determine intraspecific leaf trait variability of specific leaf area or leaf nutrient contents in forbs and trees or herbivory defence traits such as leaf silicon content in grasses (Kühn et al., 2021; Petit Bon et al., 2022; Proß et al., 2021, 2023). NIRS has also been successfully used to measure, among others, nitrogen concentration in fresh and dried leaves, which have been observed to be comparable in their reflectance spectra (Kothari et al., 2022; Kühn, Proß, et al., 2024). Kühn, Proß, et al. (2024) in particular have tested the influence of herbarium storage on plant specimens and found no significant deviations in reflectance characteristics between recently dried samples and those who have been exposed to real or simulated herbarium storage. As the NIRS method is economical, non-destructive and has fast sample processing times, it has the potential to unlock the value of herbarium specimens for extracting ecological data that covers decades and even centuries without altering the specimen.

Therefore, the present study aims at employing non-destructive spectroscopy to detect long-term changes in tissue nutrients, especially nitrogen (N), carbon (C), phosphorus (P) content and the resulting ratios of carbon:nitrogen (C:N) and nitrogen:phosphorus (N:P), in large sample sizes covering arable fields and mesic meadows over a longer period of at least several decades. By visiting the herbarium records, resurveying locations and analysing contemporary samples, we tested the following hypotheses:

1. At the regional scale, we predict the concentrations of leaf nitrogen and leaf phosphorus content to increase over time as captured by herbarium specimens.
2. We predict leaf nitrogen and leaf phosphorus concentrations to be higher in arable fields than in mesic meadows due to more intensive management practices on arable fields and also to display larger changes compared to their historic samples.
3. We hypothesized that continued high input of nitrogen coupled with stable or even declining addition of phosphorus fertilizer during the late 20th century induced an increase in leaf nitrogen while leaf phosphorus contents lag behind, resulting in increasing N:P ratios.

2 | METHODS

2.1 | Experimental set-up

To test these hypotheses, a resurvey study was carried out in 2022 in two regions in central Germany located around the cities of Görlitz and Jena. Görlitz is located in the German federal state of Saxony, close to the Polish border at 51°9′19″ N, 14°59′19″ E; while Jena is in Thuringia and lies at 50°55′44″ N 11°34′00″ E. These two cities possess extensive herbarium collections: the Senckenberg Museum for Natural History herbarium (GLM) comprises more than 350,000 specimens in total, mainly collected in Upper Lusatia and neighbouring areas of Saxony. The Senckenberg Herbarium Haussknecht in Jena (JE) houses around 3.5 million specimens and is one of the largest European herbaria. Both collections date back to the 19th century and contain specimens that are even older. Furthermore, both cities are located in the foothills of German intermediate mountain ranges (*Mittelgebirge*), the surrounding areas are dominated by agriculture and forestry, and both were formerly part of the German Democratic Republic.

We selected arable fields and mesic meadows as study systems because they account for more than half of the land cover in Germany today (Hemmerling et al., 2021), can harbour a large number of species, and are habitats that are created and maintained by human activity. To select species for the study of arable fields and mesic meadows, we used extensive lists with plant species frequency in vegetation samples compiled by Wesche et al. (2012) and Meyer et al. (2013), and we selected the 50 most frequent species from each habitat type. Taxonomy was standardized by cross-referencing with the *Rothmaler Exkursionsflora von Deutschland* (Jäger, 2017). A complete list of the species included in this study can be found in the Supporting Information (Table S1).

Specimens for the 50 most frequent species, for each habitat as described by the previously mentioned studies were searched for in the herbarium. The total number of herbarium specimens available for a single species was constrained by the following selection criteria: first, the sampling information provided on the specimen label had to be precise enough to pinpoint the location in the field. Based on the location descriptions, the geographic information system QGIS, version 3.24 (QGIS Development Team, 2021) was used to relocate respective sites with maximum precision and enter them as georeferenced locations on a map (Figure 1). Satellite images provided by Google Maps (Google, 2022) were consulted to check if the habitat remained comparable to the one at time of sampling. Second, study areas of 30 km surrounding the two study cities Jena and Görlitz were established, within which the field sampling was carried out. This limited the time spent driving to field locations and made it possible to visit several locations per day.

2.2 | Resampling and specimen processing

Field sampling took place from early May to early August 2022. A maximum of five contemporary individuals per species were recollected depending on species' abundance at that site. Plant individuals at full flowering were preferred, since many of the herbarium samples were also collected in that stage. Furthermore, this reduced the impact of seasonal variations in leaf traits (Römermann et al., 2016). Field sampling was then carried out according to standard herbarium protocols: the stem of the plant was cut off just below the soil surface and the plant material was placed in a newspaper sheet, along with a paper slip denoting sample identity and data. The plant stem was bent at sharp angles if the plant would not fit onto the sheet otherwise. The wrapped plant was then placed in a portable field press for fixation and pre-drying of specimens. If a species could not immediately be found at a given location, 15 min were allotted to search for it. If the species was not found in that time, or the search would have led into a different habitat, the species was noted as absent. Conversely, individuals from our species list were sampled at field locations even if there was no corresponding historical herbarium specimen. The 'full' dataset thus includes samples which are field samples not connected to specific herbarium samples or vice versa, while the 'paired' dataset consists of a historical herbarium specimens and corresponding recent samples taken from the same species at the same location. A list of the locations included in this study can be found in the Supporting Information (Table S2). At the end of each day of field work, samples were pressed in a plant press, placed in a drying oven at 60°C and then stored for further use.

2.3 | Laboratory analysis of leaf nutrients

Leaf material for laboratory analysis was taken from dried samples gathered in the field and then analysed following the handbook of Pérez-Harguindeguy et al. (2013). For each species at each field location, one specimen from the field was randomly selected for laboratory analysis. Plant leaves were ground to a fine powder using a grinder mill (MM 400, Retsch, Haan, DE). Five milligrams of leaf powder per sample was used for the analysis of carbon–nitrogen content (Vario EL Cube, Elementar Analysensysteme, Langenselbold, DE), while for the analysis of leaf phosphorus content, a nitric acid digestion was carried out on 200 mg of leaf powder per sample using a Loftfield device. The liquified samples were then analysed for phosphorus content using an ion chromatograph (ICS-90 Dionex, Thermo Fischer Scientific, Waltham, USA).

2.4 | Spectroscopic analysis of leaf nutrients

All leaf samples were subjected to near-infrared spectroscopy, using an ASD Field Spec 4 Wide-Res (Malvern Panalytical Ltd., Almelo, Netherlands), which captured a spectrum from 350 to 2500 nanometres (nm). In a first step, spectral data were gathered on all dried leaf samples, including historic herbarium specimens and recently sampled and pressed field specimens. For this, a Field Spec probe with a 2 mm diameter was placed on the

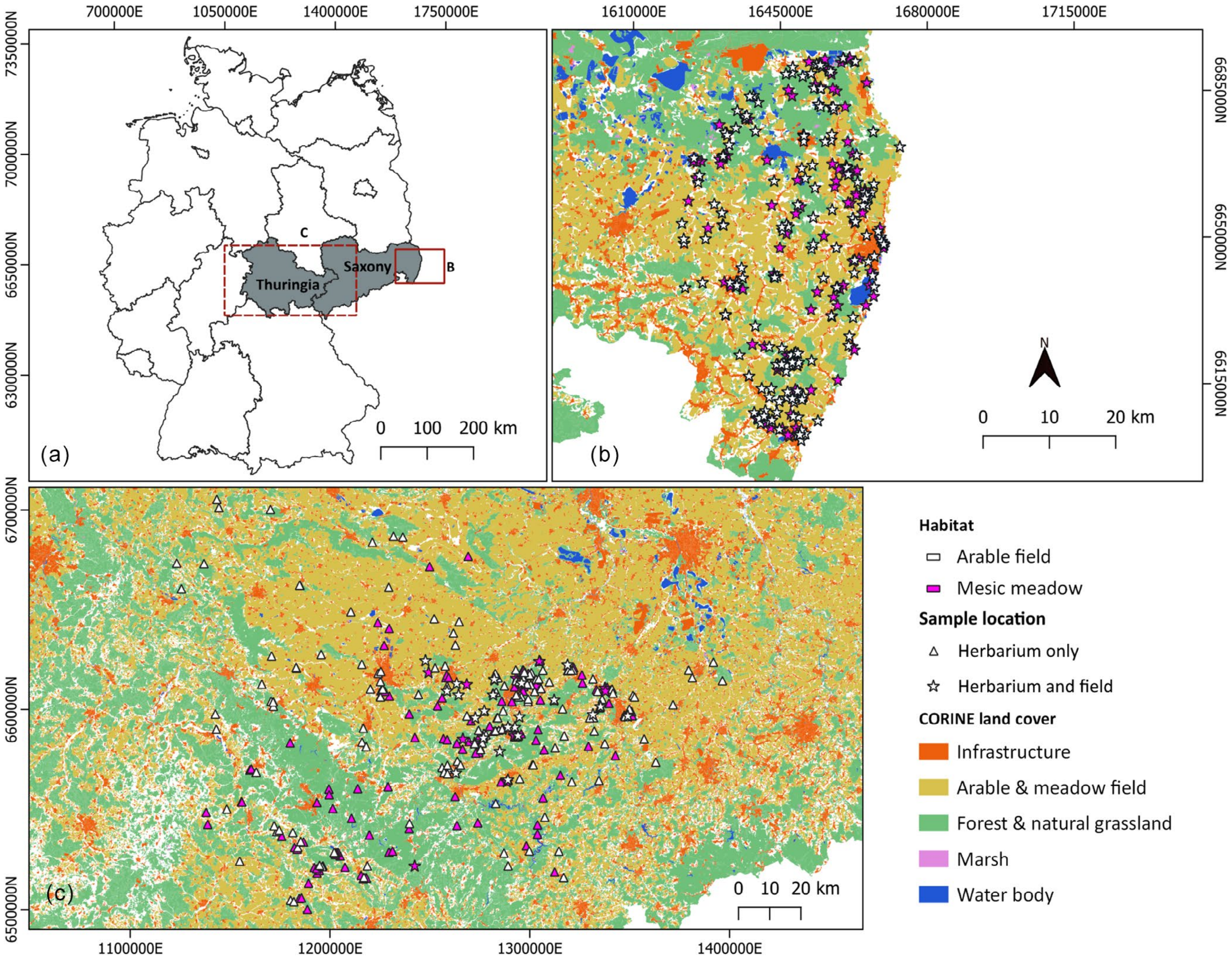

FIGURE 1 Maps of field sites showing (a) the location of the two federal states of Saxony and Thuringia within Germany, (b and c) the sampling locations within Thuringia and Saxony, respectively, with corresponding habitat types and CORINE land cover classes (European Environment Agency, 2019). Each point represents the sampling location of either one herbarium specimen (triangles) or of a herbarium specimen and corresponding field specimens (stars).

adaxial side and at the widest part of the leaf. Three spectral readings were taken from the same spot successively and then averaged for further use. The Field Spec was regularly calibrated using a white reference (Zenith Lite Target, Sphere Optics GmbH, Herrsching, Germany).

The laboratory and spectral data from the recent, dried samples was then used to build a calibration model. Previous studies have found that once dried, leaf characteristics between recently conserved samples and older herbarium specimens remain comparable (Kühn, Proß, et al., 2024). For the calibrations, the workflow from Proß et al. (2023) and Kühn, Proß, et al. (2024, see Supporting Information) was used. This workflow utilizes the programming language R (R Core Team, 2021) and the '*plantspec*' package (Griffith & Anderson, 2019) to carry out a partial least squares regression (PLSR) for each trait. The trait-specific calibration models can then be used to predict trait data from spectral data alone, which was then done for both the herbarium specimens and the field samples. Where necessary, outliers in the calibration were pruned and reference values were transformed using the natural logarithm to reshape them to facilitate the calculation of the PLSR model. Calibration models were created for the trait values for which laboratory data were present. For the ratios of carbon to nitrogen and nitrogen to phosphorus, values were calculated based on the predictions results from the calibration models of the single constituent traits. Model quality was assessed using the root mean square error of prediction (RMSEP) and the coefficient of determination R^2.

2.5 | Historical variation in N and P fertilization

Based on publicly available records in the statistical yearbooks of the German *Reich* (1925–1940), German Democratic Republic (1949–1989) and Federal German Republic (1994–2022), a dataset was compiled which listed the annual level of N and P fertilizer use in kg per hectare per year (Staatliche Zentralverwaltung für Statistik, 1955–1990; Statistisches Bundesamt, 1976–2021; Statistisches Reichsamt, 1880-1941/1942). The fertilization data records are currently published on a federal state level (Saxony and Thuringia), which does not allow for finer distinctions such as between habitat types. In the GDR, these metrics were tracked on a district level, with the GDR districts Gera, Erfurt and Suhl intersecting with the studied parts of the federal state of Thuringia. The GDR district of Dresden covered all the areas sampled in and around Görlitz. The fertilizer data compiled from the statistical yearbooks covered the time from the 1920s to the present, with some gaps in the records during larger political upheavals. Generally, the amount of annual fertilizer input per unit area of arable land peaked in the 1970s for both nitrogen and phosphorus input. Nitrogen fertilizer use decreased more slowly and was less uniform across spatial scales after the 1970s peak (Figure 2).

2.6 | Statistical analysis

The resulting dataset was analysed using the R programming language (R Core Team, 2021), and "*tidyverse*" packages were used to facilitate data handling and visualization (Wickham et al., 2019).

To test our first hypothesis, whether leaf nitrogen and phosphorus content increases over time, linear mixed-effect models were calculated using the paired dataset with multiple samples from different time periods for each location. One model was created for each leaf trait (leaf nitrogen content, leaf carbon content, leaf phosphorus content and the derived leaf carbon:nitrogen ratio, leaf nitrogen:phosphorus ratio), with the given trait as dependent variable, and year and habitat type as covariates alone as well as in interaction with each other. Random effects were included in the model, with the different sampling locations being nested within the two study regions. To test for the influence of functional groups, species were identified as either a

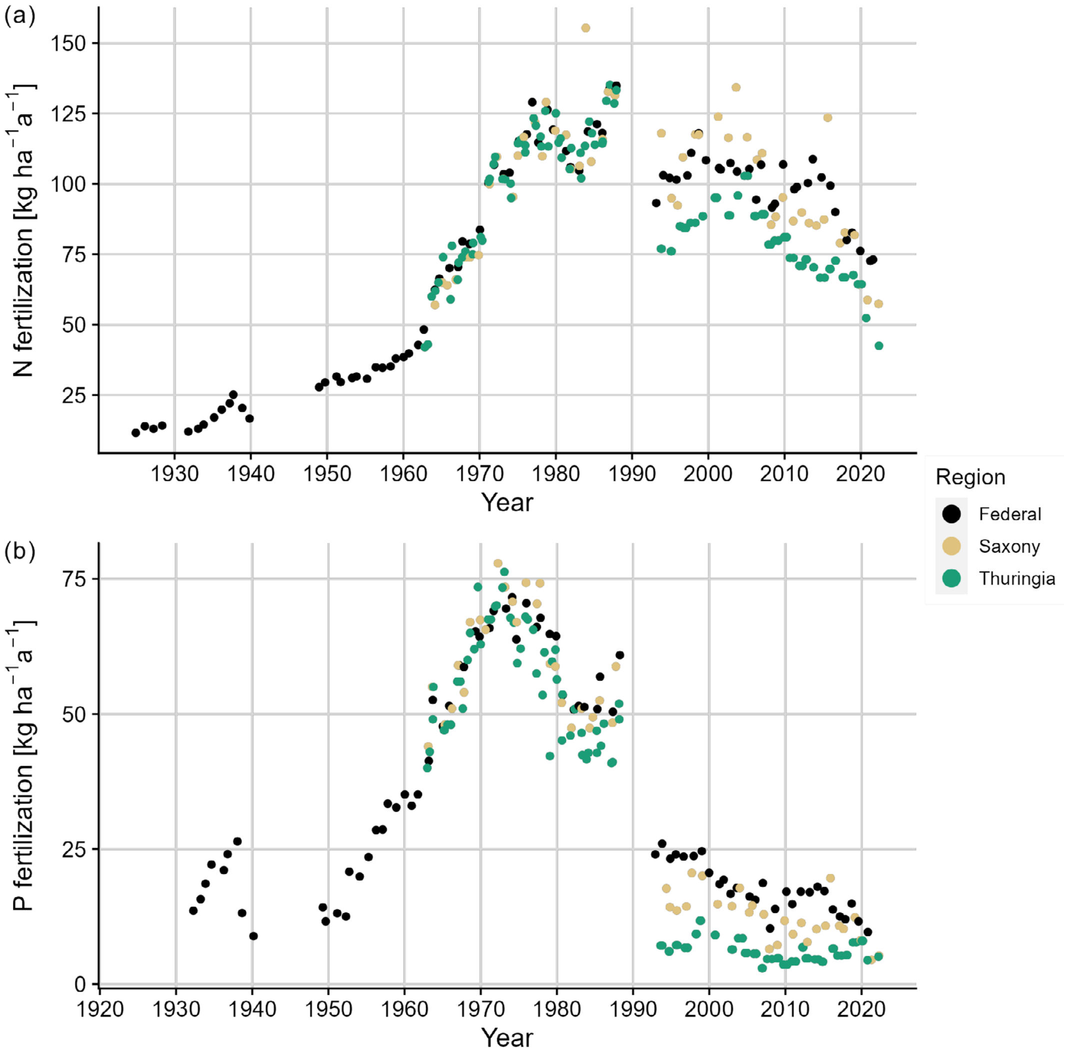

FIGURE 2 Amount of (a) N and (b) P fertilizer in kg per hectare applied per year over time. Colours represent the different spatial scales, with black representing the country-wide fertilizer input; khaki, the fertilizer input in the territory of the state of Saxony; and green, the fertilizer input on the territory of the state of Thuringia. Tracking of fertilizer input on the scale of individual federal states only began in the 1960s.

forb, a legume or a grass based on the family identity (Table S1). An additional species-level analysis was carried out for a dataset comprising the 10 most common species, each of which had more than 10 samples in the paired dataset (Table S3). For the models, no random effects were added to avoid overfitting or singular fits. The model calculation on the species level was followed by a post-hoc Bonferroni adjusted t-test as implemented by the R package '*emmeans*' (Lenth, 2023) to determine if the trends of the 10 most common species over the years differed significantly from each other.

For our second hypothesis, in which we propose that leaf nitrogen and phosphorus values from mesic meadow specimens are lower than those from arable field specimens, we calculated log response ratios from the smaller, paired dataset. We did this by dividing values for leaf nitrogen content, leaf phosphorus content and the leaf N:P ratio from the 2022 field samples by those of the historical samples and taking the natural logarithm of the resulting values. In cases where there were multiple field samples from one species from one location, the trait values were averaged. Linear models were created for the log response ratio with the habitat type and year as covariates alone and in interaction with each other. The year in this case can be understood as a variable that denotes the time span between the 'control' of the herbarium sample and the 'treatment' of the 2022 field sample.

The third hypothesis, stating that increased fertilization over time leads to increasing leaf nitrogen values while concurrent lagging phosphorus fertilization leads to rising N:P ratios, was tested using generalized additive mixed models (GAMMs) to account for non-linear covariate effects (Hastie & Tibshirani, 1986). For the GAMMs, the '*mgcv*' package was used (Wood, 2023). We used the paired dataset and created GAMMs with either leaf nitrogen content or leaf phosphorus content as dependent variables, and thin plate spline-based, penalized smoothed regional annual nitrogen or annual phosphorus input as independent variables respectively. In both cases, an autocorrelation structure of order 1 was included that had the sampling year as a time covariate and a combination character string representing each sampled population as the grouping factor.

3 | RESULTS

The NIR spectroscopy was applied to a total number of 1270 individuals from 76 target plant species representing both arable field ($N=38$ species) and mesic meadow ($N=47$ species) habitats in both study regions. Out of this, 815 were specimens from the herbarium, 455 were specimens from the field and 325 specimens were pairs, consisting of one herbarium specimen and one or more field specimens of the same species sampled at the same location. The total time span covered was 172 years from 1850 to 2022, with the second half of the 20th century being especially well-represented. This matches with the most rapid changes in regional agricultural practices.

3.1 | Leaf trait calibration models

The three PLSR calibration models created from field sample spectra and laboratory analysis data differed in calibration accuracy: The calibration for leaf nitrogen content yielded an accuracy of $R^2=0.80$, with a RMSEP of 0.22. The calibration for leaf carbon content achieved an accuracy of $R^2=0.52$, with a RMSEP of 1.82. Leaf phosphorus calibrations resulted in an R^2 of 0.41 and a RMSEP of 0.79. Except for leaf carbon content, reference values were transformed using the natural logarithm to compensate for non-normally distributed reference values. The resulting models thus also predicted logarithmic leaf nitrogen, phosphorus and carbon values.

The NIRS calibrations calculated from our dataset proved to be of mixed quality. While for leaf nitrogen, the R^2 of 0.80 for measurements of full, complete dried leaves was in the same range as the literature references (Kothari et al., 2022), qualities for leaf carbon and leaf phosphorus were lower: the literature reference attained R^2 values of 0.74 and 0.61, while our models produced R^2 values of 0.52 and 0.41, respectively.

3.2 | Leaf nutrient changes over time

Leaf nutrient contents among the precise dataset changed significantly with time. Leaf nitrogen content and the leaf N:P ratio increased significantly over the years (Figure 3a,e), while leaf phosphorus content (Figure 3b), leaf carbon content (Figure 3c) and leaf C:N ratio significantly decreased (Figure 3d, Table 1). In the case of leaf nitrogen content, leaf carbon content and leaf C:N, a significant interaction was observed between the sampling year and the habitat type (Figure 3c, Table 1).

A similar pattern emerged from the species level analysis. The 10 most common species (five from each habitat, see Table S3) showed different trends over time (Figure 4). For all traits, year, species identity and their interaction term were significant (Table S4). Post-hoc tests revealed that for the leaf nitrogen content, *Agrostis capillaris* exhibited a significantly stronger increase over the years than the average, while *Polygonum aviculare* and *Potentilla reptans* exhibited a significantly weaker increase than the average, while *Consolida regalis* was the only species that exhibited a decrease in leaf nitrogen content over time. For leaf phosphorus content, among an average decrease, *Achillea millefolium*, *Consolida regalis*, *Galium mollugo* and *Polygonum aviculare* exhibited a significantly weaker decrease in this trait than the average, while for *Dactylis glomerata*, there was a significantly stronger decrease. The species that significantly differed from the average increase of the leaf N:P ratio were *Agrostis capillaris*, *Dactylis glomerata* and *Viola arvensis* with a significantly stronger increase, and *Consolida regalis* and *Polygonum aviculare* with a significantly weaker increase (Table S5). The functional groups of forb, grass and legume were found to have a significant impact on the leaf nitrogen, phosphorus and carbon content, as well as the leaf nitrogen:phosphorus ratio, both as main factors and as interaction terms with sampling year. While grasses exhibited higher leaf carbon

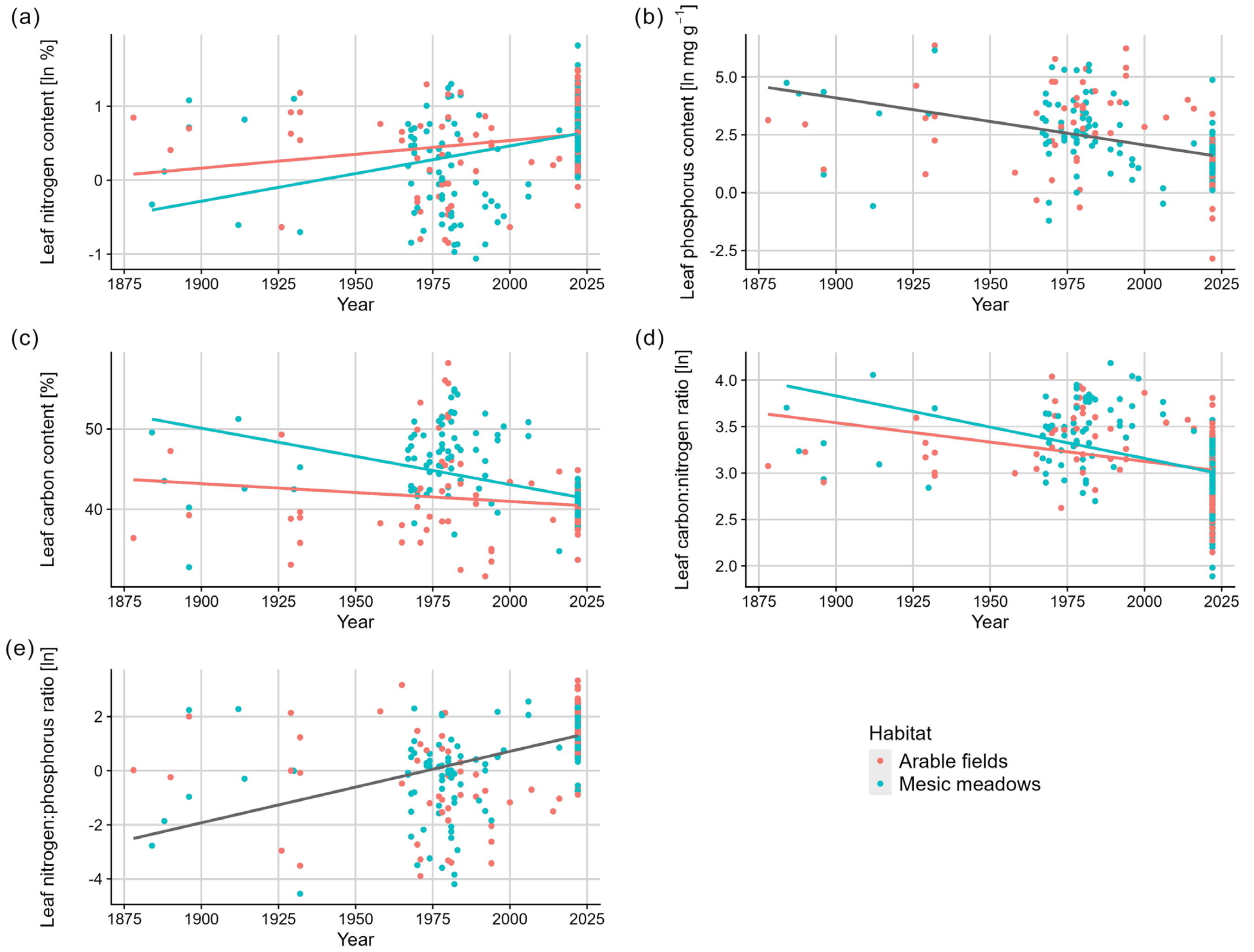

FIGURE 3 Changes in leaf traits over time as extracted from the NIRS measurements for (a) logarithmic leaf nitrogen content in %; (b) logarithmic leaf phosphorus content in mg per g; (c) leaf carbon content in %, (d) logarithmic leaf carbon:nitrogen ratio and (e) logarithmic leaf nitrogen:phosphorus ratio. Each dot represents one sample. Dot colours denote the habitat the sample was taken from or observed in, with red dots representing samples from arable fields and blue dots denoting a sample from mesic meadows. Regression lines represent significant trends: Dark grey lines indicate a significant change of the trait over time irrespective of habitat, coloured lines indicate a significant interaction between the year and habitat type. The exact statistics underlying these plots can be found in the text.

and phosphorus contents on average, legumes had the highest leaf nitrogen contents (Figure S1, Table S6).

3.3 | Trait changes in different habitats

When comparing the habitats across all years, only the leaf phosphorus content and the leaf N:P ratio were found to differ significantly between mesic meadows and arable fields (Figure 5A,C,E, Table S7). For leaf phosphorus content, the log response ratio (*lrr*) was significantly higher in mesic meadows. The *lrr* of the N:P ratio, showed a stronger decrease in mesic meadows (Figure 5D,F, Table S8). There was a significant interaction between habitat type and year for log response ratios of both the leaf phosphorus content and the N:P ratio ($F = 6.65$, $df = 107$, $p < 0.05$ and $F = 5.41$, $df = 107$, $p > 0.05$, Table S8).

3.4 | Annual fertilization and leaf nutrient contents

The results of the generalized additive mixed-effects models indicated that, accounting for the nested auto-regression structure of year and a combination of species-identity and location, the smoothed value of annual fertilizer input for nitrogen and phosphorus significantly influenced the leaf nitrogen ($F = 24.84$, $edf = 2.80$, $Ref.df = 2.80$, $p < 0.001$, also see Table S9) and leaf phosphorus content ($F = 56.78$, $edf = 1$, $Ref.df = 1$, $p < 0.001$, Table S9), respectively. The effective degrees of freedom for the GAMM involving leaf phosphorus content and annual phosphorus fertilization were equal to 1, indicating a linear fit. Plotting the leaf nutrient values predicted by the GAMM revealed an increase for leaf nitrogen content and a decrease for leaf phosphorus content over the years. Leaf nitrogen content showed a peak in the year 2022, after the highest amount of nitrogen fertilization (Figure 6a), while leaf phosphorus content displayed a peak coinciding with the time of highest phosphorus fertilization (Figure 6b).

TABLE 1 Results from linear mixed-effect models for traits of the paired dataset as a response to the fixed effects of year, habitat type and the interaction between the two.

	Fixed effects	Fixed and random effects	Year			Habitat type			Year × habitat type		
Response traits	R^2 Marg.	R^2 Cond.	den df	F	p	den df	F	p	den df	F	p
Leaf N	0.141	0.407	253.4	55.457	**<0.001**	252.74	5.312	**0.022**	252.9	5.204	**0.023**
Leaf P	0.214	0.227	288.53	85.584	**<0.001**	286.1	0.020	0.888	287.43	0.021	0.884
Leaf C	0.198	0.312	265.4	45.085	**<0.001**	263.85	13.727	**<0.001**	264.56	13.207	**<0.001**
Leaf C:N ratio	0.206	0.466	252.05	98.251	**<0.001**	251.45	5.006	**0.025**	251.57	4.937	**0.026**
Leaf N:P ratio	0.276	0.305	284.07	117.684	**<0.001**	281.86	0.894	0.345	283.06	0.876	0.350

Note: Random effects were the locality of the sample nested within the area (either Görlitz or Jena). Significant *p*-values are depicted in bold text.

4 | DISCUSSION

Using NIR calibrations on both historical and freshly collected samples, we were able to capture changes in nutrient concentrations of plant leaves across a time span of circa 170 years. We were able to confirm that there were significant trends over time, that these differed between arable fields and mesic meadows and that they were related to the level of annual fertilizer input. However, because the calibration accuracies are adequate but not exceptionally high, a margin of error has to be considered in the predicted leaf trait values, especially for leaf phosphorus content and the traits relating to it. While this limits the comparison of these trait values to reference values in the literature, it should not impact the proportional relationships of trait values within the dataset.

4.1 | Leaf nutrient contents change over time as tracked in herbarium specimens

Our results showed an increase in leaf N content and leaf N:P ratio as well as decreases in leaf carbon content, leaf C:N ratio and leaf phosphorus over time, which at least partially confirms our first hypothesis of increasing leaf nutrient concentrations over time. The trends over time for leaf nitrogen, leaf carbon and leaf C:N ratio significantly differed between arable fields and mesic meadows. For the leaf phosphorus content and the leaf N:P ratio, no significant difference could be found between the habitat types.

In their study of herbarium specimens gathered throughout the 20th century, Peñuelas and Filella (2001) observed an increase in leaf nutrient values such as nitrogen and phosphorus, but they were only able to find a significant trend for bryophytes. However, their selection of vascular species was dominated by woody shrubs and trees, which can be expected to be less directly exposed to fertilization than species of arable fields and mesic meadows in this study. We can confirm the conclusions, which were also reached by previous resurvey vegetation studies in German grasslands and arable fields (Meyer et al., 2013; Wesche et al., 2012), indicating an increasing dominance of N-demanding, competitive species, resulting in species-poor communities. Our data contradict the conclusions of McLauchlan et al. (2010) who observed a decrease and not an increase in leaf nitrogen content over the studied time period from 1876 to 2008. This could be due to differences between the Central European human-maintained habitats investigated in this study and prairie-type grasslands, where nutrient input is more strongly controlled by aerial deposition that is much smaller than full-scale fertilization.

Contrary to our expectations, the phosphorus content decreased over time, with a corresponding increase in the N:P ratio. A rising N:P ratio shows an overabundance of nitrogen in the soil, or an increasing lack of P, and could also lead to changing species

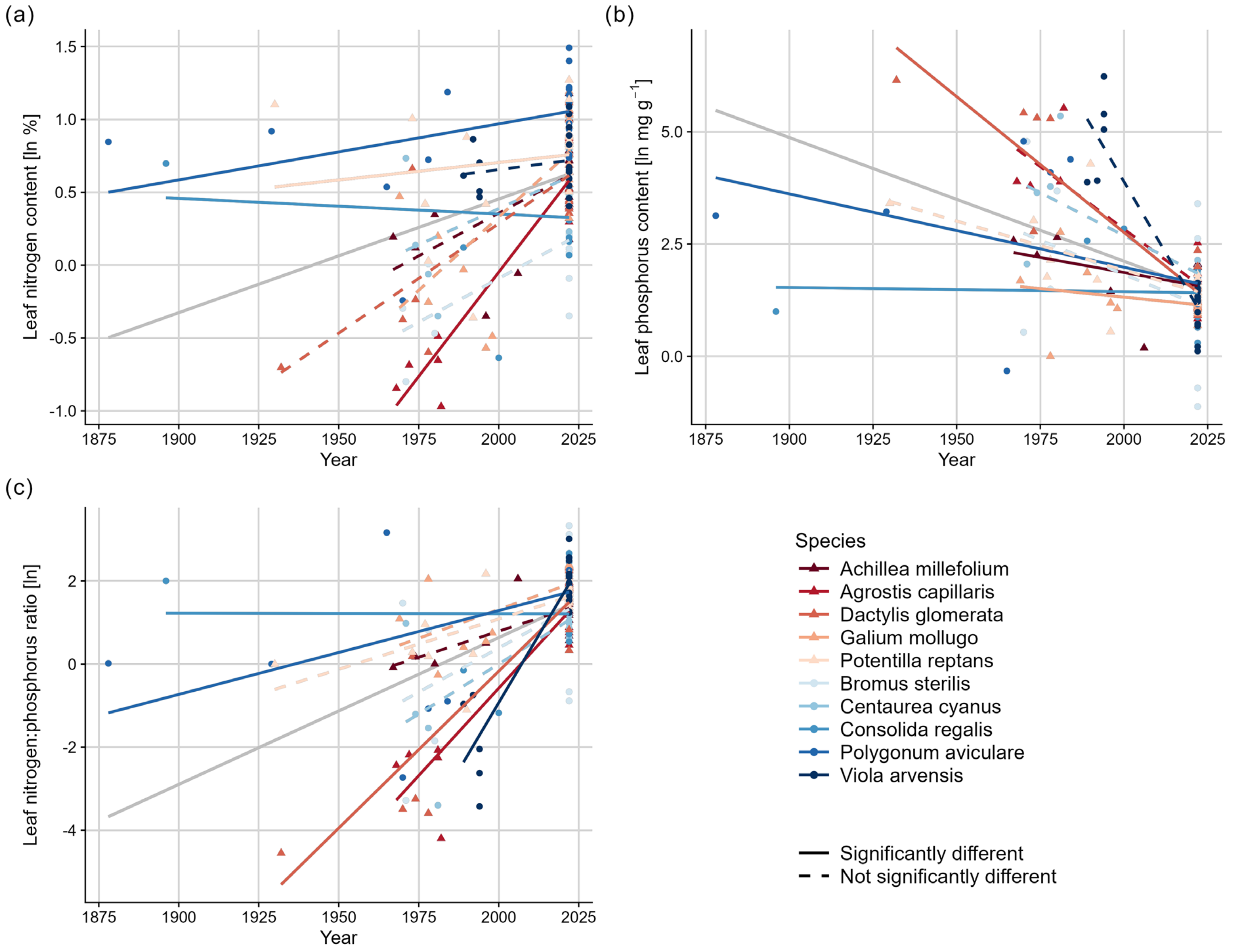

FIGURE 4 Leaf nutrient traits over the study period grouped by species for (a) leaf nitrogen content [ln %], (b) leaf phosphorus content [ln mg/g] and (c) leaf nitrogen:phosphorus ratio [ln]. Species from mesic meadows are represented with reddish colours and triangular dots, species from arable fields are represented by blueish colours and round dots. Lines represent predictions of linear models, with lines for species whose trends are significantly different from the average trend drawn solid and lines for other species drawn dashed. The grey line represents the simple linear model between Year and the respective leaf trait across all pairs for comparison.

compositions, as high N:P ratios are generally found in grass-rich, forb-poor plant communities, which are furthermore more likely to exhibit lower species richness (Güsewell, 2004).

Leaf carbon content significantly decreased over time. A higher leaf carbon content is typically an indicator of a slower, stress-tolerating growth strategy (Reich, 2014). In the context of this study, the higher carbon content could indicate that plants in mesic meadow habitats are under less pressure to shift from such conservative growth strategies compared to the faster growing, more highly fertilized arable field species (Reich, 2014).

Species-specific trends differed somewhat but without a clear pattern, except for the case of *Consolida regalis*. It is the one species in the dataset of most common species that consistently differed from the average trend in arable fields by exhibiting much slower changes, and it also is the single species among those 10 currently on the German Red List of Endangered Species (Metzing et al., 2018). Future studies could test if *Consolida regalis* is extraordinary in this regard or if there is indeed a connection between a species not benefiting from increased nutrient availability and declining populations. In the best case, long-term trait data as produced by this study could be used to augment distribution data to make more accurate forecasts of population trends possible. Functional groups significantly differed in the trends over time, with for example legumes exhibiting generally higher leaf nitrogen content values over the time, while grasses had higher leaf phosphorus content values which decreased faster than forbs over the years. However, the proportion of functional groups were not equally distributed across time spans and habitat types, which could have biased the results.

4.2 | Different responses by habitat type

The leaf nitrogen:phosphorus ratio was both generally higher in arable fields, and increased significantly faster during the studied time

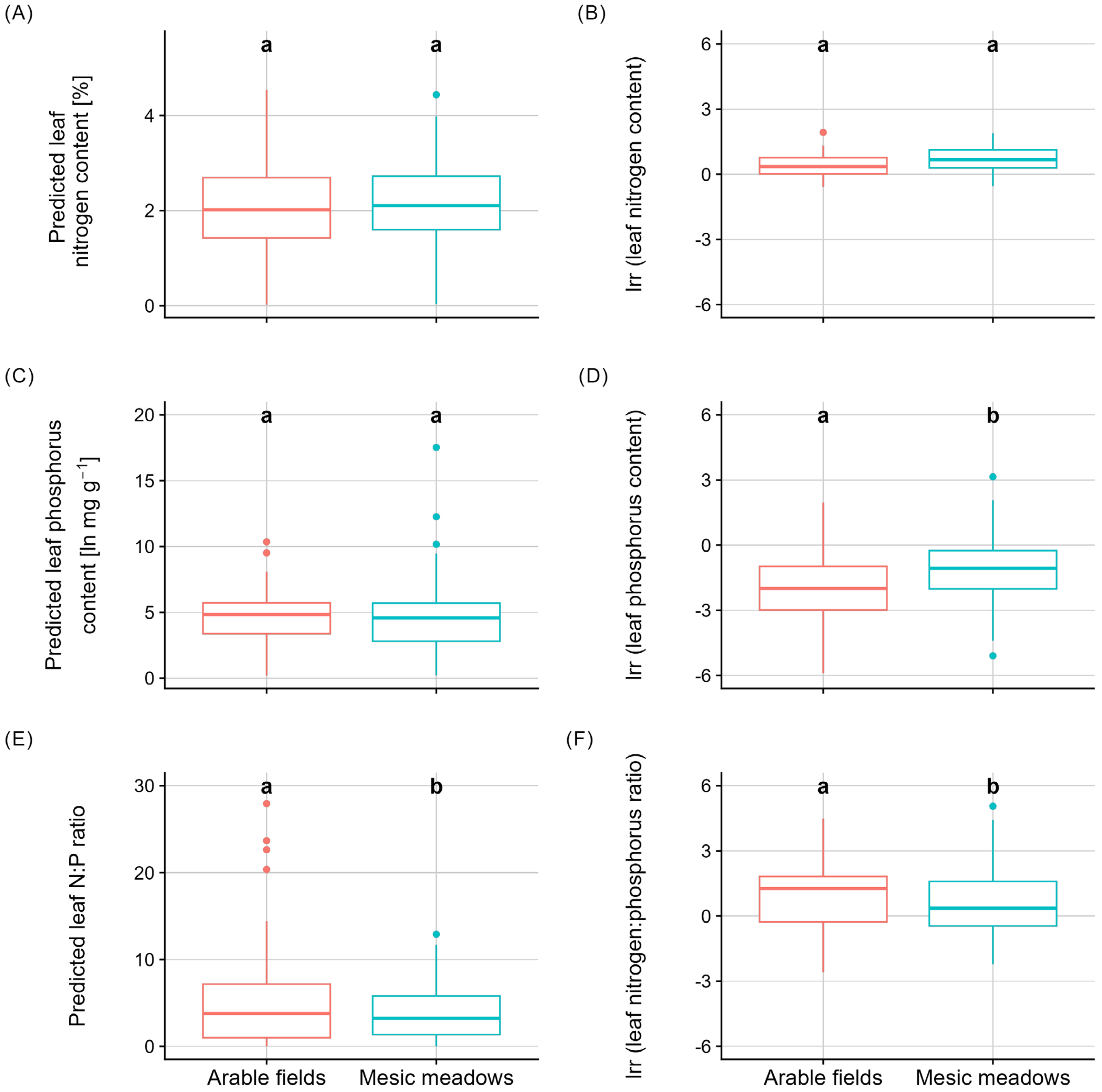

FIGURE 5 Trait values (A, C, E) and log response ratios of leaf element contents (lrr; B, D, F) of the paired dataset belonging to either arable fields (*N* = 21) or mesic meadows (*N* = 33) for leaf nitrogen content (A and B), leaf phosphorus content (C and D) and leaf nitrogen:phosphorus ratio (E and F).

period when compared to mesic meadow habitats. This partially confirms our second hypothesis that the more intensive management that arable field plants are subjected to compared to mesic meadow plants leads to higher leaf nutrient concentrations in plants from agricultural fields. However, leaf phosphorus content decreased significantly slower and the leaf N:P ratio increased significantly slower in mesic meadow habitats as indicated by the log response ratios, indicating a slower change in these traits when compared to arable fields, thereby refuting our assumption that changes would happen at the same rate in both habitats. It generally conforms with our assumption that management intensities in arable fields are more uniformly high (LfL, 2023) and would also explain why grassland habitats exhibit a slower decline in plant diversity when compared to that of arable fields: a 30%–50% decline in median plot-level species counts over 50 years in grasslands (Wesche et al., 2012) compared to a 70% loss in median plot-level species richness in arable field habitats (Meyer et al., 2013). Furthermore, it reflects the fundamental differences between mesic meadows and arable fields: as highlighted above, the former are dominated by perennial competitors, while the latter are dominated by annual ruderals.

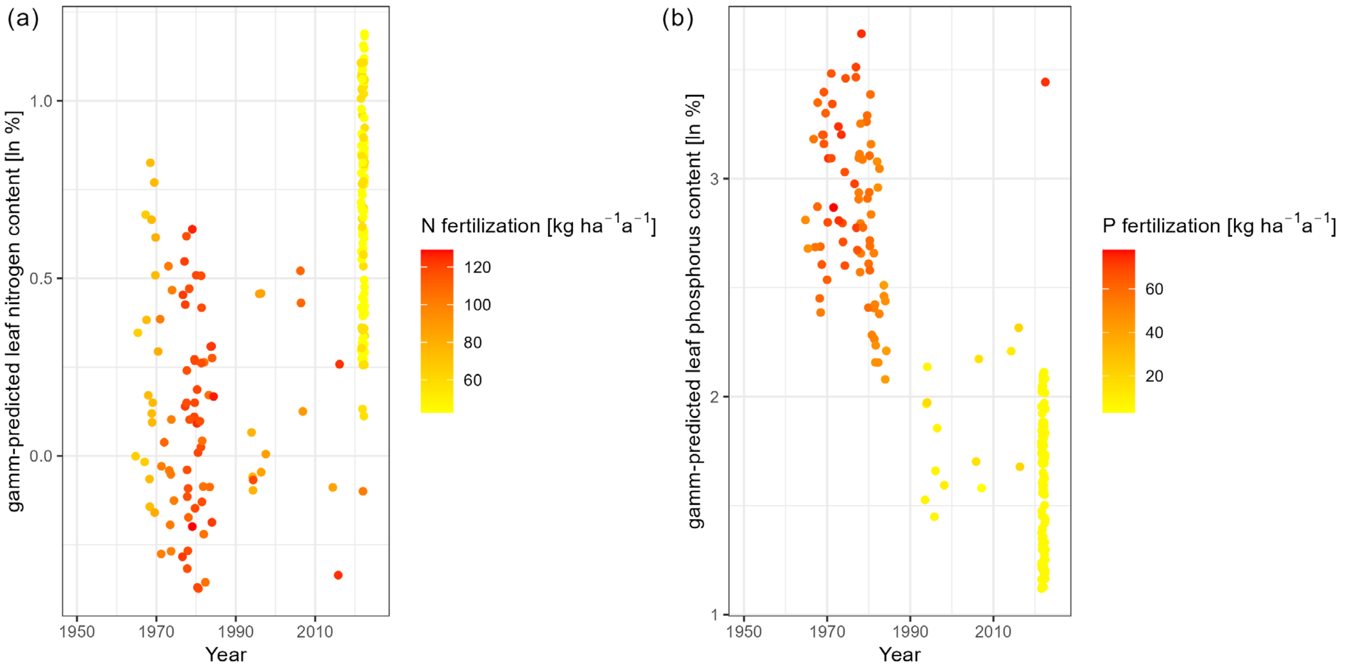

FIGURE 6 Plot showing (a) leaf nitrogen content values and (b) leaf phosphorus content values as predicted by the GAMM for each fertilization level. While the year was not involved in describing the GAMM model, it is used here as the *x*-axis for orientation. Colours denote the amount of mineral fertilizer in kilogram applied per hectare per year.

4.3 | Leaf nitrogen content tracks fertilizer input

Our results concur with those of Baessler and Klotz (2006) and, as given atmospheric inputs are only relevant with respect to N, they support our third hypothesis that phosphorus and nitrogen fertilization had a significant impact on leaf nutrient content. The level of fertilization input in both cases peaked in the 1970s and 1980s and decreased afterwards, although the decrease for nitrogen is somewhat less pronounced. This could explain why nitrogen fertilization has a significant impact on the leaf nitrogen level, but showed no interaction with year. Additionally, these dynamics can explain the observed decrease in leaf phosphorus content over time. While nitrogen fertilizer input remained at a steadily high level, phosphorus fertilizer input peaked in the 1970s and then receded to the pre-peak level, an observation also made by Baessler and Klotz (2006). The widening gap between the amount of fertilizer applied for each of these two nutrients corresponds well to the increasing N:P ratio. Furthermore, the different trends in annual fertilizer input can also help explain the results of the GAMM as plotted against the year: because the amount of annual nitrogen fertilizer used peaked in the 1970s and then remained at a high level, the GAMM model predicts a general increase of leaf nitrogen content. The amount of annual phosphorus fertilizer used however dropped sharply after the peak of the 1970s, and the GAMM correspondingly predicts a decrease in leaf phosphorus content. However, the analysis of the GAMM predictions should be carried out with care, since uncertainty from several different levels is involved here, starting from the NIRS predictions. This issue not withstanding, we can still confirm the findings of Güsewell (2004), who described a potential anthropogenic switch from P-limited to N-limited plant growth in terrestrial ecosystems induced through both direct and indirect human impacts.

5 | CONCLUSIONS

The trends observed here would indicate a general shift towards the acquisitive end of the leaf economics spectrum. Long-term fertilization and eutrophication of the soil have a clear impact on leaf nutrient traits. The apparent change from P-limited to N-limited growth that we observed corroborates this conclusion. Mesic meadows are still less intensively managed and the plants in this habitat are still turning towards the fast, nutrient-demanding growth strategies that are already present in arable field habitats. The different trends we were able to observe on a species level deliver another puzzle piece to comprehensively understand the declines in diversity that have been observed in these habitats (Baessler & Klotz, 2006; Duprè et al., 2010; Meyer et al., 2013; Wesche et al., 2012).

The results presented here also offer the possibility of future research on changes in nutrient content, for example by expanding the group of habitats selected for the study or connecting the historical trait data with historical distribution data. This can then be used to further our general understanding of long-term changes, especially anthropogenic changes, and their impacts on both natural and semi-natural plant communities.

AUTHOR CONTRIBUTIONS

All authors conceived the experimental design and methodology together. Paul Kühn and Raymond Umazekabiri carried out the field

and laboratory work, carried out the analysis of the results and led the writing of the manuscript. All authors critically contributed to the manuscript and gave their final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors state that no conflict of interest exists.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data for this paper are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.z34tmpgpw (Kühn, Umazekabiri, Römermann, Bruelheide, & Wesche, 2024).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. List of studied species with the family, the habitat type the specimen was reportedly found in, the functional group and the respective number of individuals found and included in our dataset.

Table S2. Sampling locations of herbarium specimens in the precise dataset, with their latitude and longitude in decimal degrees (WGS 84) and the habitat type.

Table S3. The ten most common species in the smaller paired dataset, based on sample numbers.

Table S4. Analysis of variance for the natural logarithm of leaf nitrogen concentration, leaf phosphorus concentration and leaf nitrogen:phosphorus ratio in relation to the interacting terms of Year and Species of the ten most common species in the dataset.

Table S5. Statistical results of post-hoc tests carried out on the models of Supporting Information 7 as calculated by the R package "*emmeans*".

Table S6. Analysis of variance for leaf nitrogen concentration, leaf phosphorus concentration as well as leaf carbon:nitrogen ratio and leaf nitrogen:phosphorus ratio in relation to the interacting terms of Year and Functional Group (defined as Grass, Forb and Legume species) of the paired dataset.

Table S7. Linear model formulas and results for the analysis of variance of the trait values (leaf nitrogen content, leaf phosphorus content and leaf N:P ratio) of herbarium samples between the arable field and mesic meadow habitat types.

Table S8. Linear model formulas and results for the analysis of variance of the log response ratio (lrr) of leaf nitrogen content, leaf phosphorus content, nitrogen:phosphorus ratio and habitat type and year.

Table S9. Formulas and results for the GAMMs as specified and output in R.

Figure S1. Changes in leaf traits over time as extracted from the NIRS measurements for (a) logarithmic leaf nitrogen content in %; (b) logarithmic leaf phosphorus content in mg per g; (c) leaf carbon content in %, (d) logarithmic leaf carbon:nitrogen ratio and (e) logarithmic leaf nitrogen:phosphorus ratio.

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