

Soil $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values are shaped by nutrient interactions rather than phosphorus alone in an 82-year fertilized grassland

Qiqi Wang^{a,b,*}, Xiong Fang^a, Jie Chen^a, Sara L. Bauke^c, Thomas F. Döring^d, Albert Tietema^e, Miaoyue Zhang^f, Roland Bol^b

^a College of Land Resources and Environment, Jiangxi Agricultural University, Nanchang 330045, PR China

^b Institute of Bio- and Geosciences, Agrosphere (IBG-3), Forschungszentrum Juelich GmbH, 52425 Juelich, Germany

^c Institute of Crop Science and Resource Conservation, Soil Science and Soil Ecology, University of Bonn, 53115 Bonn, Germany

^d Institute of Crop Science and Resource Conservation, Agroecology and Organic Farming, University of Bonn, 53121 Bonn, Germany

^e Institute of Biodiversity and Ecosystem Dynamics (IBED), University of Amsterdam, 1090 GE Amsterdam, the Netherlands

^f School of Environmental Science and Engineering, Sun Yat-sen University, Wailuan East Road, No. 132, Guangzhou 510006, PR China

ARTICLE INFO

Handling Editor: Dr. C Mueller

Keywords:

Phosphorus status
Natural element isotopes
Fertilization
Nutrient stoichiometry
Meadow grassland

ABSTRACT

Natural isotope ratios ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$) are effective indicators of soil element cycling, yet the role of phosphorus (P) in regulating their variation under long-term fertilization remains unclear. Therefore, we compared an unfertilized Control, non-P fertilized plots: receiving Ca (as lime), CaN and P-fertilized plots: CaNP, CaNPKCl, and CaNPK₂SO₄ in a meadow grassland (Rengen, Germany). Topsoil (0–10 cm) C:N:P:S range varied in line with fertilization, i.e., 109:8:1:1 (Control) to 45:4:2:1 (CaNPK₂SO₄). In the topsoil, P-fertilized plots exhibited notably lower C:nutrient ratios than the Control. In contrast, all nutrient-addition treatments significantly enriched soil $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ compared to the Ca treatment, indicating accelerated organic matter C and N turnover. Whereas isotopic responses in the subsoil were weaker. By contrast, $\delta^{34}\text{S}$ changed little across treatments except under CaNPK₂SO₄, suggesting a direct fertilizer effect. Across both depths, strong correlations ($p < 0.01$) between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ indicated tightly coupled C and N cycling, whereas $\delta^{34}\text{S}$ was unrelated to either isotope. Redundancy analysis explained 55.4% of isotopic variation in topsoil and 61.2% in subsoil. Marginal tests identified TP and TS as significant explanatory variables in both soil layers, whereas TN was additionally significant only in topsoil. These results indicate that isotopic variation was better explained by coupled nutrient interactions, particularly involving P and S, and in topsoil additionally N. In conclusion, nutrient management in this P-limited grassland should adopt a balanced strategy rather than focusing on phosphorus alone.

1. Introduction

Phosphorus (P) is essential for microbial RNA synthesis and cell membrane structure (Elser et al., 2000). Changes in phosphorus availability profoundly influence the biogeochemical cycles of carbon (C), nitrogen (N), and sulfur (S) by regulating microbial metabolic strategies, functional gene expression, and elemental stoichiometry (Kirkby et al., 2013; Zechmeister-Boltenstern et al., 2015; Spohn et al., 2016; Li et al., 2022b). Despite its key role, P limitation is widespread, affecting approximately 43% of global terrestrial ecosystems (Du et al., 2020; Wieder et al., 2015). Moreover, this proportion is increasing under intensified N deposition (Elser et al., 2007; Penuelas et al., 2020; Wang

et al., 2023), especially in low-latitude regions subjected to long-term weathering and pedogenesis, as well as in temperate grasslands impacted by N deposition (Vitousek et al., 2010; Deng et al., 2017; Fan et al., 2018).

Phosphorus limitation exhibits a dual regulatory role on C cycling. On the one hand, it reduces organic C decomposition and benefits soil C accumulation by suppressing microbial activity (Cleveland et al., 2002; Vitousek et al., 2010) and decreasing microbial carbon use efficiency (CUE) (Li et al., 2022b). On the other hand, microbial acquisition of phosphorus via phosphatase secretion is accompanied by the release of excess C (Dijkstra et al., 2013; Finn et al., 2016). The effect of P availability on the N cycle is equally complex. Phosphorus addition in P-

* Corresponding author at: College of Land Resources and Environment, Jiangxi Agricultural University, Fangzhimin Avenue, No. 1101, Jingkai District, Nanchang 330045, PR China.

E-mail address: wangqiqi@jxau.edu.cn (Q. Wang).

<https://doi.org/10.1016/j.geoderma.2026.117889>

Received 29 December 2025; Received in revised form 27 May 2026; Accepted 1 June 2026

Available online 4 June 2026

0016-7061/© 2026 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC license (<http://creativecommons.org/licenses/by-nc/4.0/>).

limited soil enhances net N mineralization rates by reducing soil N:P and C:P ratios (Reed et al., 2007; Sardans et al., 2021; Cui et al., 2022; Hu et al., 2025), but simultaneously increases the abundance of denitrification genes, thereby increasing potential gaseous nitrogen loss (Godwin and Cotner, 2015; Tang et al., 2016; Xiao et al., 2025). Phosphorus availability also indirectly affects S transformation through competitive adsorption and enzymatic activity regulation. P addition can desorb soil sulfate because phosphate has a higher affinity for soil minerals than sulfate ions (Kaiser et al., 1996). Meanwhile, phosphate competitively inhibits arylsulfatase activity (Tabatabai and Bremner, 1970; Al-Khafaji and Tabatabai, 1979), reducing organic sulfur hydrolysis and exacerbating sulfur deficiency.

Natural isotope of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ are effective in identifying the element source and soil organic matter turnover across ecosystems (Bol et al., 2000; Frank et al., 2004; Hobbie and Hobbie, 2008; Gunina and Kuzyakov, 2014; Dou et al., 2016). For instance, variations in natural $\delta^{13}\text{C}$ abundance can help to indicate the relative contributions of new and old soil organic carbon (SOC) (Bol et al., 2000; Gunina and Kuzyakov, 2014; Dou et al., 2016). $\delta^{15}\text{N}$ enrichment infers greater N cycling rates and N losses (Reis et al., 2017; Wang et al., 2021), and $\delta^{34}\text{S}$ values help indicate whether the excess S comes from desorbed sulfate or organically-cycled S (Novák et al., 2003). Given that P status is the key factor of soil C, N and S cycling, elucidating changes in natural isotopes under different P-related nutrient conditions is critical for predicting changes in terrestrial ecosystem functions (Sun et al., 2022; Feng et al., 2023). However, the effect of P status on soil natural isotopes was rarely reported. Whether P status is the primary factor influencing isotopic variation also remains unclear.

To address this gap, we investigated an 82-year fertilized grassland experiment with documented P limitation in Rengen, Germany (Hejman et al., 2010a; Hejman et al., 2010c; Smarda et al., 2013), where soils were treated with varying combinations of lime and mineral fertilizers: Ca (lime), CaN, CaNP, CaNPKCl, and CaNPK₂SO₄. All treatments without P fertilizer exhibit a negative P balance (Hejman et al., 2010a). Using ^{14}C and ^{35}S approaches, we previously found that soil P (indicated by soil total P content) deficits decrease microbial CUE and S bioavailability, particularly in N-fertilized plots without P (CaN), whereas P fertilization can alleviate microbial P limitation and enhance soil S mineralization rates (Wang et al., 2024). Based on these findings, we, therefore, hypothesized that P status (indicated by soil total P content or stoichiometry) is a primary driver of soil natural isotope ratios ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$). Specifically, higher P availability enhances microbial decomposition and organic matter turnover (Mehnaz et al., 2019; Xu et al., 2020; Wang et al., 2024), leading to an enrichment of natural isotopes in P-fertilized soils compared to P-deficient treatments.

2. Material and methods

2.1. Experiment site

The fertilizer experiment was established at the Rengen Grassland Research Station of the University of Bonn in the Eifel Mountains (Germany, 50°14'22"N, 6°49'34"E). The site is 475 m above sea level, the annual average temperature and total precipitation of 6.9 °C and 811 mm (mean value, 1934–1991, Rengen meteorological station), respectively. The soil type is Stagnic Cambisol (IUSS working group WRB, 2022).

In 1941, two blocks were set up in the trial site and each block has 25 plots (3 m × 5 m). Five different fertilizer treatments (Ca, CaN, CaNP, CaNPKCl, and CaNPK₂SO₄) with five replicates each were randomly dispersed in each block (Fig. S1 for field photography and design). The nutrients are applied as the following fertilizers: quick lime, limestone ammonium nitrate (KAS 27), potassium chloride, basic slag and potassium sulfate (Smarda et al., 2013). The annual application amount of nutrients for each treatment are as follows (average inputs in kg ha⁻¹ yr⁻¹): Ca (715 Ca), CaN (752 Ca, 100 N), CaNP (936 Ca, 100 N, 35P),

CaNPKCl (936 Ca, 100 N, 35P, 133 K), CaNPK₂SO₄ (936 Ca, 100 N, 35P, 133 K). These fertilizers were applied in a single annual dressing except for N (two dressings). Since the original field experiment did not contain non-fertilized controls, an unfertilized control treatment was introduced on a strip next to the existing block in 1988. The experimental plots were mown once a year in late summer from 1942 to 1944 and 1950–1961 for hay production, the experiment was not managed (fertilized or cut) during 1945–1950. Since 1962, all plots have been mowed twice a year, in late June/early July and mid-October. From 1989 until 1995, forage biomass was measured by cutting the sward in each plot to ca. 2 cm (Schellberg et al., 2001). More information on aboveground plant species and trial details have been published previously (Hejman et al., 2007; Hejman et al., 2010c).

2.2. Soil sampling

In November 2022, soil samples were collected from five fertilization treatments in block 1 and the control (Fig. S1). Specifically, after removing plant residues, 6 auger cores (Ø 3 cm) were taken with a thickness of 0–10 cm and 10–20 cm in each plot, then soil samples were combined and mixed thoroughly to form a single composite sample for each depth (i.e. topsoil and subsoil) in each plot, with a total of 60 samples collected (6 treatments, 5 replicates and 2 depths). Soil samples were transported to the laboratory immediately and stored at – 4 °C before further analysis. After removing all stones and roots, soil samples were air-dried and sieved to a size of 2 mm and 0.15 mm for the determination of soil properties.

2.3. Soil abiotic properties and natural isotope determination

Soil pH was measured using a soil-to-water ratio of 1:2.5. Soil total calcium (Ca), magnesium (Mg), aluminium (Al), iron (Fe), potassium (K), manganese (Mn), total sulfur (TS) and total phosphorus (TP) were measured by inductively coupled plasma optical emission spectroscopy (Thermo Scientific ICP-OES, Waltham MA, USA) after acid digestion (approximately 50 mg per sample) was performed in closed vessels in a Microwave system using a mixture of 2 mL concentrated HCl and 0.7 mL concentrated HNO₃.

To remove the inorganic carbon, the SOC and total nitrogen (TN) contents, the $^{13}\text{C}/^{12}\text{C}$ ratios and $^{15}\text{N}/^{14}\text{N}$ isotope ratios were determined by dry combustion on an isotope ratios mass spectrometer (Thermo-Finnigan, Bremen, Germany) after decalcification with hydrochloric acid (5% HCl). Briefly, samples were decalcified by wet chemical treatment. About 150 mg of each sample was weighed in centrifuge tubes, treated with 25 mL of hydrochloric acid (HCl, 5%) and kept in a 50 °C water bath for 4 h. Samples were washed and centrifuged repeatedly with 45 mL of deionized water, to remove the remnants of the hydrochloric acid and then dried and homogenized for isotope analysis (Pötter et al., 2020). The isotope ratios were represented as $\delta^{13}\text{C}$ (‰) and $\delta^{15}\text{N}$ (‰) relative to the Vienna Pee Dee Belemnite (VPDB) international standard ($^{13}\text{C}_{\text{VPDB}}$) and atmospheric air ($^{15}\text{N}_{\text{air}}$). The $^{34}\text{S}/^{32}\text{S}$ isotope ratios in soil samples were measured by isotope ratio mass spectrometry (vision, Elementar Analysensysteme GmbH, Langenselbold, Germany) using samples without HCl treatment and expressed as $\delta^{34}\text{S}$ relative to the Vienna Canyon Diablo meteorite (VCDT) standard. Measurements were calibrated using the international reference substances silver sulfide (IAEA-S1 and IAEA-S2) and barium sulfate (IAEA-SO-6).

2.4. Statistical analysis

One-way ANOVA was conducted to evaluate the effect of fertilization on soil properties and natural isotope values using SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Significant differences among treatments were determined using Duncan test at the $p < 0.05$ level. Data were tested for homogeneity of variance using Levene's test ($p > 0.05$) prior to ANOVA. When the data did not meet the assumptions

of normality or homogeneity of variances, the Kruskal-Wallis H test ($p < 0.05$) was used to assess treatment differences. Independent Samples *t*-test was used for the comparison between topsoil and subsoil within each fertilization treatment ($p < 0.05$). Pearson correlations and redundancy analysis (RDA) were performed in R 4.2.1 using the ggplot2 and vegan packages, respectively. RDA was applied to examine the relationships between isotopic composition ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$) and soil properties (SOC, TN, TP, TS, K, Ca, Mg, Fe, Al, Mn, and pH). Nutrient stoichiometric ratios were excluded to avoid deterministic redundancy. All variables were centered and scaled prior to analysis. Model significance was assessed using 999 permutations, and marginal tests were used to evaluate the significance of each explanatory variable after accounting for the others. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Soil element content

Fertilization had a strong effect on belowground soil nutrient stocks in this grassland. In topsoil, soil pH values in limed soils (7–7.6) were significantly increased relative to the control (5.4 ± 0.18). Moreover, 82-year lime application significantly decreased SOC content (Table 1). Specifically, the SOC decreased by 16% on average under Ca and CaN, and remarkably decreased by 34% on average under treatments with P fertilizers (CaNP, CaNPKCl, CaNPK₂SO₄). Soil TP content was higher under treatments with P fertilizers (CaNP, CaNPKCl, CaNPK₂SO₄, 1.40 g kg⁻¹ on average) compared to treatments without P fertilizers (Control, Ca, CaN, 0.41 g kg⁻¹ on average). Soil TS content increased by 38% on average under Ca (0.53 ± 0.02 g kg⁻¹) and CaN (0.52 ± 0.02 g kg⁻¹), and increased by 65% on average under treatments with P fertilizers relative to control (0.38 ± 0.02 g kg⁻¹). Moreover, fertilization significantly increased soil total Mg content relative to control, total Fe content was significantly higher under treatments with P fertilizers than in treatments without P fertilizers (Table 2).

In the subsoil, soil pH in limed subsoil also significantly increased (Table 1). Fertilization had no significant effect on SOC and TN. Soil TP

Table 1
Soil pH and nutrient content under different treatments.

Depths (cm)	Treatments	Soil pH	SOC (g kg ⁻¹)	TN (g kg ⁻¹)	TP (g kg ⁻¹)	TS (g kg ⁻¹)
0–10	Control	5.36 ± 0.18c	42 ± 3.85 a	3.07 ± 0.22 a	0.43 ± 0.03c	0.38 ± 0.02 d
		7.30 ± 0.15 a	34 ± 1.4 bc	2.92 ± 0.09 a	0.43 ± 0.03c	0.53 ± 0.02c
	Ca	7.32 ± 0.09 a	36 ± 2.7 ab	2.85 ± 0.08 a	0.36 ± 0.01c	0.52 ± 0.02c
		7.60 ± 0.14 a	24 ± 2.3 d	2.74 ± 0.27 a	1.58 ± 0.14 a	0.65 ± 0.05 ab
	CaNP	7.03 ± 0.25b	28 ± 1.5 cd	2.67 ± 0.14 a	1.25 ± 0.10b	0.56 ± 0.04 bc
		7.49 ± 0.04 a	30 ± 1.8 bcd	2.95 ± 0.08 a	1.36 ± 0.09 ab	0.67 ± 0.05 a
	CaNPKCl	5.46 ± 0.19b	18 ± 1.45 a	1.50 ± 0.15 a	0.39 ± 0.03b	0.23 ± 0.03b
		7.26 ± 0.08 a	20 ± 2.8 a	1.61 ± 0.19 a	0.35 ± 0.03b	0.32 ± 0.03 ab
	CaN	7.20 ± 0.04 a	20 ± 2.7 a	1.92 ± 0.19 a	0.31 ± 0.01b	0.35 ± 0.03 a
		7.56 ± 0.04 a	20 ± 2.0 a	1.94 ± 0.27 a	0.83 ± 0.11 a	0.42 ± 0.08 a
	CaNPK ₂ SO ₄	7.20 ± 0.23 a	22 ± 2.4 a	1.86 ± 0.21 a	0.86 ± 0.11 a	0.36 ± 0.36 a
		7.35 ± 0.10 a	21 ± 0.7 a	1.91 ± 0.15 a	0.78 ± 0.04 a	0.37 ± 0.02 a
10–20	Control	5.46 ± 0.19b	18 ± 1.45 a	1.50 ± 0.15 a	0.39 ± 0.03b	0.23 ± 0.03b
		7.26 ± 0.08 a	20 ± 2.8 a	1.61 ± 0.19 a	0.35 ± 0.03b	0.32 ± 0.03 ab
	Ca	7.20 ± 0.04 a	20 ± 2.7 a	1.92 ± 0.19 a	0.31 ± 0.01b	0.35 ± 0.03 a
		7.56 ± 0.04 a	20 ± 2.0 a	1.94 ± 0.27 a	0.83 ± 0.11 a	0.42 ± 0.08 a
	CaNP	7.20 ± 0.23 a	22 ± 2.4 a	1.86 ± 0.21 a	0.86 ± 0.11 a	0.36 ± 0.36 a
		7.35 ± 0.10 a	21 ± 0.7 a	1.91 ± 0.15 a	0.78 ± 0.04 a	0.37 ± 0.02 a
	CaNPKCl	5.46 ± 0.19b	18 ± 1.45 a	1.50 ± 0.15 a	0.39 ± 0.03b	0.23 ± 0.03b
		7.26 ± 0.08 a	20 ± 2.8 a	1.61 ± 0.19 a	0.35 ± 0.03b	0.32 ± 0.03 ab
	CaN	7.20 ± 0.04 a	20 ± 2.7 a	1.92 ± 0.19 a	0.31 ± 0.01b	0.35 ± 0.03 a
		7.56 ± 0.04 a	20 ± 2.0 a	1.94 ± 0.27 a	0.83 ± 0.11 a	0.42 ± 0.08 a
	CaNPK ₂ SO ₄	7.20 ± 0.23 a	22 ± 2.4 a	1.86 ± 0.21 a	0.86 ± 0.11 a	0.36 ± 0.36 a
		7.35 ± 0.10 a	21 ± 0.7 a	1.91 ± 0.15 a	0.78 ± 0.04 a	0.37 ± 0.02 a

SOC: soil organic carbon, TN: soil total nitrogen, TP: soil total phosphorus, TS: soil total sulphur. The different lower cases indicate significant differences among treatments in topsoil (0–10 cm) or subsoil (10–20 cm) ($p < 0.05$). Data are represented as means ± SEM.

Table 2

Soil total metal content under different treatments.

Depths (cm)	Treatments	Ca (g kg ⁻¹)	Mg (g kg ⁻¹)	Al (g kg ⁻¹)	Fe (g kg ⁻¹)	K (g kg ⁻¹)	Mn (g kg ⁻¹)
0–10	Control	1.38 ± 0.27b	1.10 ± 0.02 d	16 ± 0.38 ab	20 ± 0.44b	1.98 ± 0.07 a	1.41 ± 0.10b
		3.81 ± 0.24 ab	1.74 ± 0.04 bc	15 ± 0.22b	19 ± 1.33b	1.78 ± 0.10 a	1.33 ± 0.10b
	Ca	3.75 ± 0.22 ab	1.78 ± 0.07b	16 ± 0.39 ab	20 ± 1.08b	1.80 ± 0.10 a	1.39 ± 0.13b
		6.45 ± 0.75 a	1.99 ± 0.09 a	16 ± 0.41 a	25 ± 1.11 a	1.92 ± 0.08 a	1.94 ± 0.05 a
	CaNP	3.63 ± 0.33 ab	1.58 ± 0.05c	16 ± 0.61 ab	24 ± 1.05 a	2.33 ± 0.26 a	1.99 ± 0.10 a
		4.85 ± 0.32 a	1.79 ± 0.05b	17 ± 0.28 ab	23 ± 0.58 a	2.26 ± 0.18 a	1.86 ± 0.07 a
	CaNPKCl	0.86 ± 0.19b	1.02 ± 0.02 a	17 ± 0.22 a	26 ± 1.33 a	2.34 ± 0.05 a	1.80 ± 0.14 a
		2.19 ± 0.34 ab	1.37 ± 0.06 a	15 ± 0.21 ab	22 ± 1.52 a	2.38 ± 0.22 a	1.62 ± 0.03 a
	CaN	1.68 ± 0.21 ab	1.02 ± 0.06 a	11 ± 0.57b	16 ± 0.60 a	2.13 ± 0.19 a	1.06 ± 0.07 a
		3.04 ± 0.49 a	1.31 ± 0.11 a	14 ± 0.96 ab	20 ± 1.81 a	2.40 ± 0.12 a	1.33 ± 0.13 a
	CaNPK ₂ SO ₄	2.56 ± 0.47 ab	1.46 ± 0.13 a	15 ± 0.79 ab	25 ± 2.51 a	2.82 ± 0.28 a	1.75 ± 0.14 a
		1.93 ± 0.69 ab	0.86 ± 0.30 a	9 ± 2.79 ab	14 ± 5.05 a	1.94 ± 0.29 a	1.12 ± 0.33 a
10–20	Control	0.86 ± 0.19b	1.02 ± 0.02 a	17 ± 0.22 a	26 ± 1.33 a	2.34 ± 0.05 a	1.80 ± 0.14 a
		2.19 ± 0.34 ab	1.37 ± 0.06 a	15 ± 0.21 ab	22 ± 1.52 a	2.38 ± 0.22 a	1.62 ± 0.03 a
	Ca	1.68 ± 0.21 ab	1.02 ± 0.06 a	11 ± 0.57b	16 ± 0.60 a	2.13 ± 0.19 a	1.06 ± 0.07 a
		3.04 ± 0.49 a	1.31 ± 0.11 a	14 ± 0.96 ab	20 ± 1.81 a	2.40 ± 0.12 a	1.33 ± 0.13 a
	CaNP	2.56 ± 0.47 ab	1.46 ± 0.13 a	15 ± 0.79 ab	25 ± 2.51 a	2.82 ± 0.28 a	1.75 ± 0.14 a
		1.93 ± 0.69 ab	0.86 ± 0.30 a	9 ± 2.79 ab	14 ± 5.05 a	1.94 ± 0.29 a	1.12 ± 0.33 a
	CaNPKCl	0.86 ± 0.19b	1.02 ± 0.02 a	17 ± 0.22 a	26 ± 1.33 a	2.34 ± 0.05 a	1.80 ± 0.14 a
		2.19 ± 0.34 ab	1.37 ± 0.06 a	15 ± 0.21 ab	22 ± 1.52 a	2.38 ± 0.22 a	1.62 ± 0.03 a
	CaN	1.68 ± 0.21 ab	1.02 ± 0.06 a	11 ± 0.57b	16 ± 0.60 a	2.13 ± 0.19 a	1.06 ± 0.07 a
		3.04 ± 0.49 a	1.31 ± 0.11 a	14 ± 0.96 ab	20 ± 1.81 a	2.40 ± 0.12 a	1.33 ± 0.13 a
	CaNPK ₂ SO ₄	2.56 ± 0.47 ab	1.46 ± 0.13 a	15 ± 0.79 ab	25 ± 2.51 a	2.82 ± 0.28 a	1.75 ± 0.14 a
		1.93 ± 0.69 ab	0.86 ± 0.30 a	9 ± 2.79 ab	14 ± 5.05 a	1.94 ± 0.29 a	1.12 ± 0.33 a

Ca: total calcium, Mg: total magnesium, Al: total aluminium, Fe: total iron, K: total potassium, Mn: total manganese. The lower cases indicate significant differences among treatments in topsoil (0–10 cm) or subsoil (10–20 cm) ($p < 0.05$), data are represented as means ± SEM.

content with P addition increased from 0.34 to 0.82 g kg⁻¹ (on average) relative to treatments without P fertilizer (Table 1). Relative to the control (0.23 ± 0.03 g kg⁻¹), all fertilization increased soil TS significantly except for the treatment Ca. Fertilization had no significant effect on total metal content in the subsoil (Table 2).

3.2. Soil nutrient stoichiometry

In the topsoil, treatments Control and CaN had significantly higher C:N ratios than treatments with P fertilizer addition (CaNP, CaNPKCl, CaNPK₂SO₄). The soil C:N:P:S ratios (Table 3) under fertilization were 109:8:1:1 (Control), 63:5:0.8:1 (Ca), 70:6:0.7:1 (CaN), 37:4:2:1 (CaNP), 51:5:2:1 (CaNPKCl), 45:4:2:1 (CaNPK₂SO₄). Fertilization had no significant effect on C:N in the subsoil. The C:N:P:S stoichiometry ratios in subsoil were 77:7:2:1 (Control), 61:5:1:1 (Ca), 58:5:0.9:1 (CaN), 47:5:2:1 (CaNP), 62:5:2:1 (CaNPKCl), 56:5:2:1 (CaNPK₂SO₄). Notably, P fertilizer addition (CaNP, CaNPKCl, CaNPK₂SO₄) significantly decreased C:P, C:S, N:S and N:P ratios and increased P:S compared to treatments without P fertilizer in two depths (Table 3).

Table 3

Stoichiometric ratios of soil elements under different treatments.

Depths (cm)	Treatments	C:N	C:P	C:S	N:S	N:P	P:S	C:N:P:S
0–10	Control	13.5 ± 0.43 a	98 ± 11 ab	110 ± 8 a	8.1 ± 0.46 a	7.3 ± 0.69 a	1.1 ± 0.05b	109:8:1:1
	Ca	11.5 ± 0.49 bc	79 ± 4b	63 ± 1b	5.6 ± 0.25b	7.0 ± 0.43 a	0.81 ± 0.05c	63:5:0.8:1
	CaN	12.7 ± 0.82 ab	101 ± 6 a	70 ± 3b	5.5 ± 0.14b	8.0 ± 0.29 a	0.69 ± 0.02c	70:6:0.7:1
	CaNP	8.8 ± 0.39 d	15 ± 1 d	33 ± 1 d	3.8 ± 0.27 d	1.7 ± 0.13c	2.2 ± 0.11 a	37:4:2:1
	CaNPKCl	10.6 ± 0.44c	23 ± 2c	51 ± 4c	4.9 ± 0.39 bc	2.2 ± 0.14b	2.3 ± 0.13 a	51:5:2:1
	CaNPK ₂ SO ₄	10.1 ± 0.45 cd	22 ± 1c	45 ± 3c	4.5 ± 0.36 cd	2.2 ± 0.16 bc	2.0 ± 0.04 a	45:4:2:1
10–20	Control	11.9 ± 0.42 a	45 ± 3 a	79 ± 6 a	6.58 ± 0.35 a	3.84 ± 0.32 abc	1.75 ± 0.16b	77:7:2:1
	Ca	12.0 ± 0.56 a	60 ± 1 a	63 ± 8 ab	5.16 ± 0.44 bc	4.73 ± 0.69 ab	1.13 ± 0.11c	61:5:1:1
	CaN	10.6 ± 0.64 a	54 ± 7 a	67 ± 7 ab	6.38 ± 0.58 ab	6.26 ± 0.66 a	1.06 ± 0.16c	58:5:0.9:1
	CaNP	10.5 ± 0.69 a	21 ± 2 bc	43 ± 6c	4.08 ± 0.44c	1.94 ± 0.16 bc	2.10 ± 0.12 ab	47:5:2:1
	CaNPKCl	12.0 ± 0.66 a	25 ± 1 ab	55 ± 7 bc	4.51 ± 0.41c	1.87 ± 0.15c	2.42 ± 0.13 a	62:5:2:1
	CaNPK ₂ SO ₄	11.0 ± 0.49 a	27 ± 1 ab	57 ± 2 bc	5.23 ± 0.32 bc	2.45 ± 0.15 abc	2.14 ± 0.08 ab	56:5:2:1

C:N: soil organic carbon to total nitrogen ratio, C:P: soil organic carbon to total phosphorus ratio, C:S: soil organic carbon to total sulphur ratio, N:S: total nitrogen to total sulphur ratio, N:P: total nitrogen to total phosphorus ratio, P:S: soil total phosphorus to total sulphur ratio. The different lower cases indicate significant differences among treatments in topsoil (0–10 cm) or subsoil (10–20 cm) ($p < 0.05$). Data are represented as means $\pm$ SEM.

3.3. Soil natural isotopes values

The range of natural $\delta^{13}\text{C}$ was from -28.72‰ to -28.29‰ in topsoil (Fig. 1). The combination of lime (Ca) with N, P or S fertilizers had significantly higher $\delta^{13}\text{C}$ value than sole lime application (Ca). Soil $\delta^{15}\text{N}$ value under fertilization was between 1.66‰ to 2.48‰ (Fig. 1). Compared with the control, treatments CaN, CaNP and CaNPKCl significantly increased $\delta^{15}\text{N}$. As for the $\delta^{34}\text{S}$ (Fig. 1), only CaNPK₂SO₄ ($6.58\text{‰} \pm 0.33$) significantly increased $\delta^{34}\text{S}$ compared with the control ($4.16\text{‰} \pm 0.21$).

Overall, in the subsoil, fertilization had no significant effect on the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Treatment CaNPK₂SO₄ ($6.53\text{‰} \pm 0.11$) had significantly higher $\delta^{34}\text{S}$ than the Ca ($4.57\text{‰} \pm 0.33$), CaN ($4.98\text{‰} \pm 0.20$) and CaNP ($5.07\text{‰} \pm 0.35$). Except for the CaNPKCl, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the subsoil were significantly higher than in the topsoil.

3.4. Relationships between soil nutrient contents, stoichiometry and stable isotope values

In topsoil and subsoil, natural $\delta^{13}\text{C}$ values were positively correlated with $\delta^{15}\text{N}$ values with a higher r value (0.59, 0.64 respectively, $p < 0.001$), while soil natural $\delta^{34}\text{S}$ value had no significant relationship with $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values (Fig. 2).

In topsoil, there was a negative correlation between $\delta^{15}\text{N}$ values and SOC ($r = -0.53$, $p = 0.003$), TN ($r = -0.47$, $p = 0.009$), C:N ($r = -0.39$, $p = 0.035$) and C:P ($r = -0.37$, $p = 0.048$), C:S ($r = -0.38$, $p = 0.042$) ratio (Fig. 3). Soil natural $\delta^{34}\text{S}$ values were positively correlated with

soil TS ($r = 0.38$, $p = 0.04$), total Calcium ($r = 0.37$, $p = 0.047$) and soil pH ($r = 0.38$, $p = 0.042$, Fig. S2), negatively correlated with C:P ratio and C:S ratio (Fig. 3). In the subsoil (Fig. 4), soil $\delta^{13}\text{C}$ values were negatively correlated with SOC ($r = -0.45$, $p = 0.015$) and TN content ($r = -0.5$, $p = 0.006$). Soil $\delta^{15}\text{N}$ values were positively correlated with C:S ($r = 0.43$, $p = 0.019$) and N:S ratio ($r = 0.51$, $p = 0.005$), and negatively correlated with soil SOC, TN, TP and TS content (Fig. 4).

The RDA model explained 55.4% of the isotopic variation in topsoil (adjusted $R^2 = 0.266$, $p = 0.024$) and 61.2% in subsoil (adjusted $R^2 = 0.360$, $p = 0.005$), indicating a stronger multivariate structure in subsoil (Fig. 5). Marginal tests (Table 4) identified TP and TS as significant explanatory variables in both soil layers, whereas TN was significant only in topsoil. Specifically, TP ($p = 0.019$), TS ($p = 0.010$), and TN ($p = 0.034$) significantly explained isotopic variation in topsoil, while in subsoil only TP ($p = 0.037$) and TS ($p = 0.005$) remained significant. The RDA biplots further suggested that isotopic variation in both soil layers was structured mainly along a nutrient-related gradient, with P representing an important explanatory factor together with S, and additionally N in topsoil.

4. Discussion

4.1. Soil element content and stoichiometry under different fertilization

Long-term fertilization strongly altered soil nutrient status and stoichiometry, especially in topsoil. The markedly higher C:P ratios under treatments without P addition support previous evidence that this

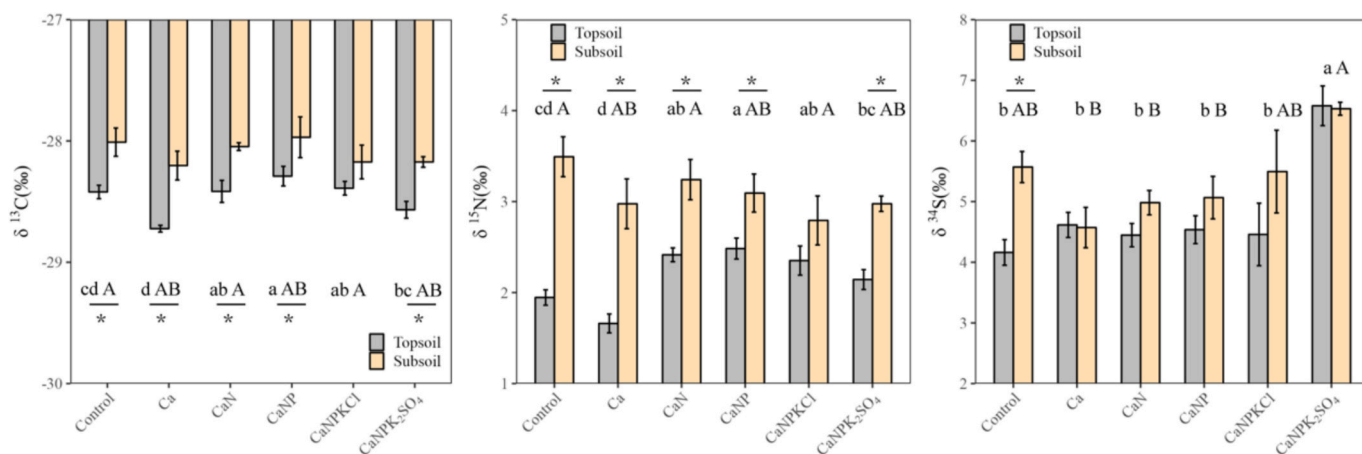


Fig. 1. Natural $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ abundance in soil samples under different treatments. The lower and upper cases above the bars indicate significant differences among these treatments in topsoil and subsoil ($p < 0.05$), respectively. * represents significance between topsoil and subsoil at $p < 0.05$. Data are represented as Means $\pm$ SEM ($n = 5$).

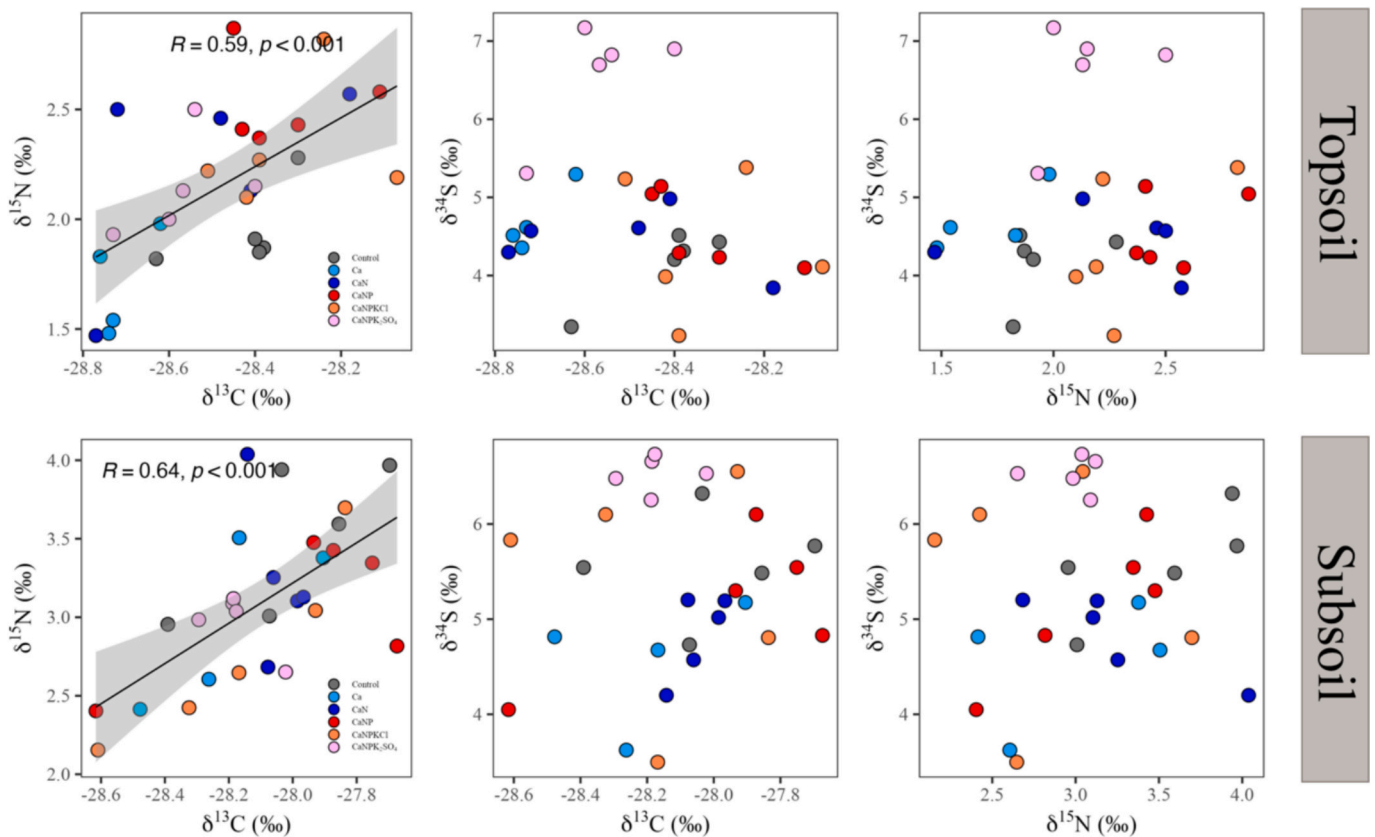


Fig. 2. The correlations between natural $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ abundance in topsoil and subsoil. r value represents the correlation coefficient.

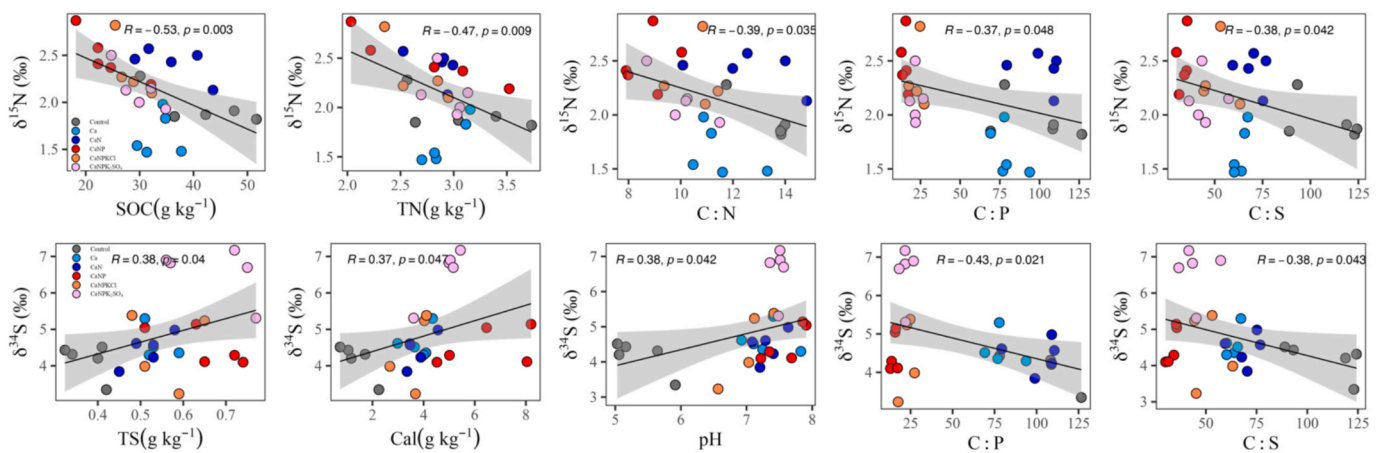


Fig. 3. The correlation relationship between natural $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$ values and soil abiotic properties in topsoil. SOC: soil total organic carbon, TN: soil total nitrogen, TS: soil total sulphur, C:N: soil organic carbon to total nitrogen ratio, C:P: soil total organic carbon to total phosphorus ratio, C:S: soil total organic carbon to total sulphur ratio, Cal: soil total calcium. r value represent the correlation coefficient.

grassland is P-limited (Chytrý et al., 2009; Hejerman et al., 2010b; Smarda et al., 2013). By contrast, P fertilization substantially increased TP and shifted soil C:N:P:S stoichiometry toward lower C:P, N:P, C:S and N:S ratios and higher P:S ratios, indicating pronounced nutrient reorganization under long-term fertilization (Sun et al., 2022). Similar effects of fertilization on soil nutrient pools and stoichiometry have been widely reported (Luo et al., 2020; Rocci et al., 2022; Wang et al., 2022a). Different from the topsoil, fertilization had no significant effect on the C:N ratios in the subsoil. This is likely because the effects of liming or fertilization on soil properties and microbial properties are often restricted to the topsoil (Kriszan et al., 2009; Vieira Fontoura et al.,

2019; Ferrari Neto et al., 2021).

In our study, P fertilizer addition significantly reduced soil SOC, which has been reported by Sochorová et al. (2016), this is consistent with previous finding that alleviation of microbial P limitation can enhance C loss due to the higher microbial mineralization of high-quality organic inputs after P fertilization (Freschet et al., 2013; Li et al., 2022a). Moreover, soil S pool significantly increased in treatments combining lime and P fertilizer (Table 1). This suggests that fertilization modified not only P status but also the coupling between P and S pools. This agrees with previous work at this site indicating enhanced P availability, grass biomass production and S turnover rates under P

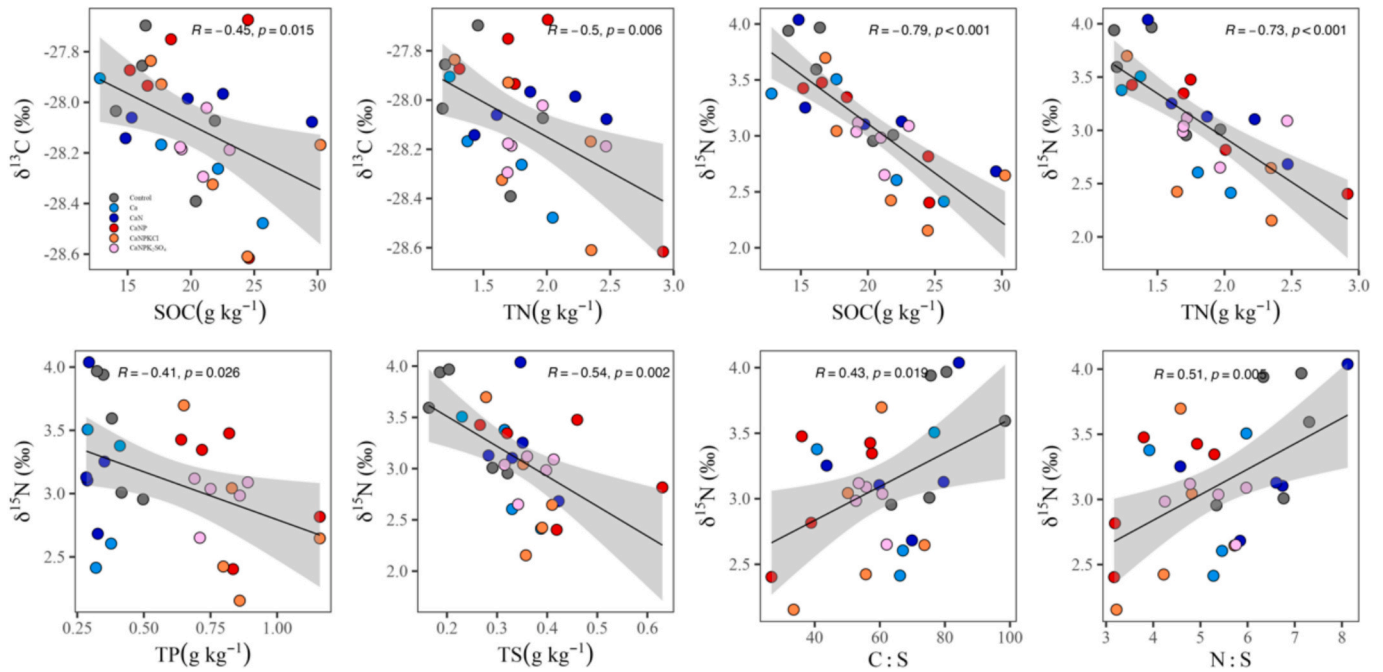


Fig. 4. The correlation relationship between natural $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$ values and soil abiotic properties in the subsoil. SOC: soil total organic carbon, TN: soil total nitrogen, TP: soil total phosphorus, TS: soil total sulphur, C:S: soil total organic carbon to total sulphur ratio, N:S: soil total nitrogen to total sulphur ratio. r values represent the correlation coefficient.

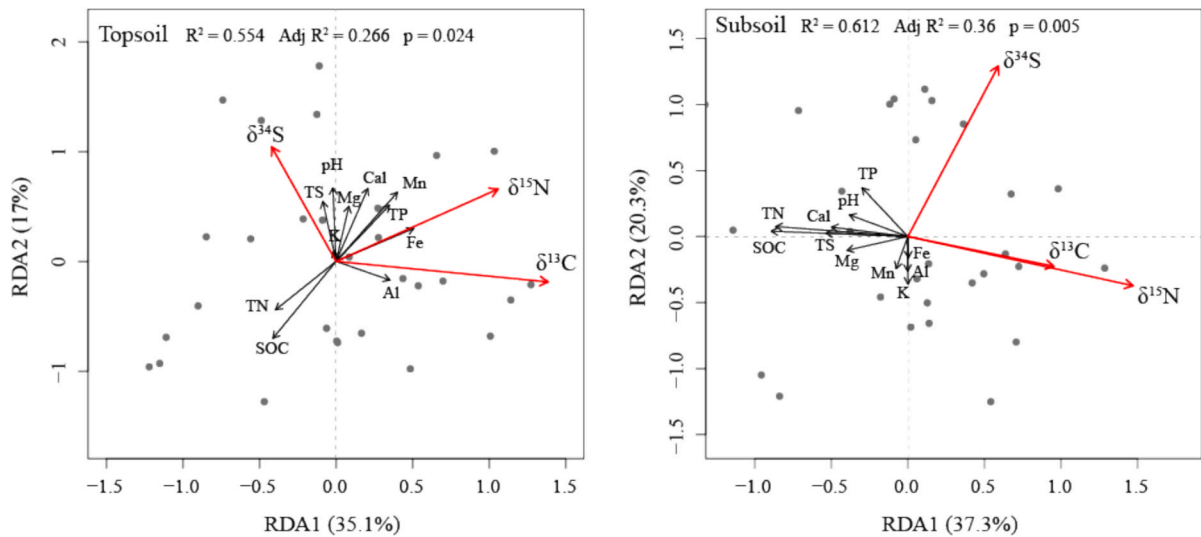


Fig. 5. Redundancy analysis (RDA) showing the relationships between soil natural isotopic composition ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$) and soil properties in topsoil and subsoil under 82-year fertilization. Black arrows represent soil variables: SOC: soil total organic carbon, TN: soil total nitrogen, TP: soil total phosphorus, TS: soil total sulphur, Cal: soil total calcium, Mg: total magnesium, Al: total aluminium, Fe: total iron, K: total potassium, Mn: total manganese. Red arrows represent isotopic variables. The direction and length of arrows indicate the strength and orientation of their relationships with the ordination axes.

fertilizer addition (Hejman et al., 2010a; Hejman et al., 2010c; Wang et al., 2024).

4.2. Soil natural isotope ratios ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$) after different fertilization

Lime (Ca) alone caused little change in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values relative to the control, indicating that the variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ under nutrient-added treatments was driven mainly by nutrient inputs (N, P or S), instead of the elevated soil pH. Fertilization type and amount both significantly affected the enrichment of soil $\delta^{15}\text{N}$ (Watzka et al., 2006; Kriszan et al., 2014). Because $\delta^{15}\text{N}$ in mineral N fertilizer or calcium

ammonium nitrate were near 0 ‰ or −1 ‰ (Kriszan et al., 2009; Choi et al., 2017), the direct contribution of mineral fertilizers to soil $\delta^{15}\text{N}$ was negligible, therefore, the observed higher $\delta^{15}\text{N}$ values were likely attributed to enhanced organic matter turnover than to direct fertilizer isotopic signatures (Bol et al., 2005; Senbayram et al., 2008; Liu et al., 2016; Liu et al., 2018). By contrast, $\delta^{34}\text{S}$ values showed minimal variation across treatments and depths except in CaNPK₂SO₄, which showed increased $\delta^{34}\text{S}$ values presumably derived from the fertilizer input, in agreement with Bol et al. (2005), who found that microbial fractionation of S isotopes is often relatively small.

Globally, a general trend of decreasing C:N and increasing $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values with depth has been observed in various ecosystems (Werth

Table 4

Marginal effects of soil properties on isotopic composition ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$) in topsoil and subsoil based on redundancy analysis (RDA).

Soil depth	Variables	Margin Variance	Margin F	Margin P
Topsoil	SOC	0.12	1.47	0.224
	TN	0.24	3.06	0.034
	TP	0.31	4.00	0.019
	TS	0.39	4.99	0.010
	K	0.21	2.63	0.058
	Cal	0.11	1.36	0.276
	Mg	0.08	0.97	0.414
	Fe	0.06	0.74	0.504
	Al	0.16	2.01	0.134
	Mn	0.10	1.24	0.328
	pH	0.07	0.91	0.421
Subsoil	SOC	0.10	1.44	0.227
	TN	0.05	0.72	0.538
	TP	0.24	3.46	0.037
	TS	0.38	5.50	0.005
	K	0.20	2.88	0.057
	Cal	0.06	0.83	0.508
	Mg	0.07	0.96	0.388
	Fe	0.13	1.93	0.150
	Al	0.14	1.98	0.132
	Mn	0.17	2.44	0.097
	pH	0.01	0.09	0.961

and Kuzyakov, 2010; Liu et al., 2016; Han et al., 2020). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ enrichment with soil depths were linked to isotope fractionation during microbial decomposition of organic matter (Hobbie and Quimette, 2009; Han et al., 2020; Gurmessa et al., 2022). The finding in the subsoil that both ^{13}C and ^{15}N were negatively correlated with SOC and TN content supported the assumption.

4.3. Is phosphorus status the primary driver of soil $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ values under long-term fertilization?

Although previous work at this site showed that soil TP content was a key factor regulating soil microbial growth and S cycling (Wang et al., 2024), our results indicate that P status was an important but not exclusive driver of isotopic variation. After accounting for other soil properties, P contributed independently to the joint variation of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ in both topsoil and subsoil (Fig. 5; Table 4). However, TS was also significant in both layers, and TN was additionally significant in the topsoil. This suggests that the isotopic response is structured along a nutrient related gradient rather than being controlled by TP alone. The strong correlation between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ confirms their coupling, which is consistent with the view that soil C and N coupling is independent of ecosystem type and soil depth (Yang et al., 2010; Han et al., 2020; Park et al., 2023). This further suggests that organic matter is the dominating reservoir of C and N in this grassland. In contrast, the natural $\delta^{34}\text{S}$ values showed no significant relationships with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, rather, they were positively correlated with TS, total Calcium, suggesting that the turnover of S in the soil is less associated with organic matter turnover and is governed by different factors.

Our findings support the hypothesis that P-fertilized and unfertilized soils induce isotopic shifts primarily through coupled nutrient cycling. This interpretation is consistent with the view that $\delta^{15}\text{N}$ integrates N transformation and loss pathways across soils and ecosystems (Bachmann et al., 2026). Previous work has shown that P supply can increase soil nitrogen transformation rates and N retention (Wang et al., 2022b), while long-term fertilization can modify P lability through changes in soil C:N:P stoichiometry (Qaswar et al., 2019). In our study, the concurrent significance of TP and TS, further suggests that P did not act in isolation, but rather within a coupled P and S nutrient framework, which agrees with our previous work that soil S cycling can respond to P availability (Wang et al., 2024). The crucial role of S wheel in driving multi-element cycling has been highlighted recently (Cheng et al.,

2025). We conclude that P acts as a key explanatory factor within a nutrient regulated system, particularly in concert with S and with N in the topsoil, but does not serve as the dominant control over isotopic variation.

4.4. Study limitations

In our study, the isotope data of fertilizers were not available, which limited our ability to separate direct fertilizer signals from post-input biogeochemical fractionation. In addition, phosphorus status was indicated by total P content and P related stoichiometry rather than directly measured available P or P fractions, so our interpretation reflects P-related nutrient status rather than individual P pools (Qaswar et al., 2019; Wang et al., 2024). Finally, RDA models explained part of the isotopic variation, suggesting that isotopic variation was likely influenced not only by the measured soil properties but also by other environmental controls, including climate, atmospheric deposition, and microbial processes (Seal, 2006; Peri et al., 2012; Nehlich, 2015; Shan et al., 2019).

5. Conclusion

Long-term fertilization significantly altered soil nutrient status, stoichiometry, and natural isotope composition in this grassland, with stronger effects in topsoil than in the subsoil. Phosphorus fertilization increased soil TP and shifted C:N:P:S stoichiometry, but the variations of soil $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ values were not explained by P status alone. Instead, RDA showed that isotopic variation was structured by nutrient interactions, particularly involving TP and TS in both soil layers, and additionally TN in topsoil. These results indicate that P status is an important, but not primary, driver of soil isotope patterns in this 82-year fertilized grassland. From a management perspective, although this grassland was indicated as a P-limited system, a balanced nutrient strategy should be considered rather than focusing on P alone. Stoichiometric ratios may serve as useful indicators of nutrient imbalance and long-term soil functional change in grasslands. Additionally, variables related to soil S should be included for long-term monitoring in addition to N and P, with particular attention to topsoil organic carbon conservation.

CRedit authorship contribution statement

Qiqi Wang: Writing – review & editing, Writing – original draft, Visualization, Validation, Formal analysis, Data curation, Conceptualization. **Xiong Fang:** Writing – review & editing, Visualization. **Jie Chen:** Writing – review & editing, Visualization. **Sara L. Bauke:** Writing – review & editing, Visualization, Supervision, Resources, Data curation. **Thomas F. Döring:** Writing – review & editing, Visualization, Resources. **Albert Tietema:** Writing – review & editing, Visualization, Supervision. **Miaoyue Zhang:** Writing – review & editing, Visualization, Funding acquisition. **Roland Bol:** Writing – review & editing, Visualization, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

We gratefully acknowledge the financial supported from Helmholtz Association POF IV: 2021-2026, grant 2173 “Towards a Sustainable Bioeconomy – Resources, Utilisation, Engineering and Agroecosystems” (Germany) and National Natural Science Foundation of China (NSFC) Sino-German Mobility Programme (No. M-0749). The first author Qiqi

Wang acknowledges the Ph.D grant from the China Scholarship Council (No.201903250108).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.geoderma.2026.117889>.

Data availability

Data will be made available on request.

References

- Al-Khafaji, A., Tabatabai, M., 1979. Effects of trace elements on arylsulfatase activity in soils. *Soil Sci.* 127 (3), 129–133.
- Bachmann, M., Tian, Y., Heinze, J., Borken, W., Inselsbacher, E., Wanek, W., Schindlbacher, A., 2026. Changes in natural ^{15}N abundance highlight warming-induced stimulation of soil nitrate losses by coupled nitrification–denitrification in an old-growth montane forest. *Geoderma* 467, 117746.
- Bol, R., Amelung, W., Friedrich, C., Ostle, N., 2000. Tracing dung-derived carbon in temperate grassland using ^{13}C natural abundance measurements. *Soil Biol. Biochem.* 32 (10), 1337–1343.
- Bol, R., Eriksen, J., Smith, P., Garnett, M.H., Coleman, K., Christensen, B.T., 2005. The natural abundance of ^{13}C , ^{15}N , ^{34}S and ^{14}C in archived (1923–2000) plant and soil samples from the Askov long-term experiments on animal manure and mineral fertilizer. *Rapid Commun. Mass Spectrom.* 19 (22), 3216–3226. <https://doi.org/10.1002/rcm.2156>.
- Cheng, T., Wang, Y., Jones, D.L., Bol, R., Wanek, W., Liang, Y., Ma, Q., 2025. Sulphur wheel: a neglected driver for the coupling of multi-element cycles in soil. *Biol. Fertil. Soils*. <https://doi.org/10.1007/s00374-025-01968-4>.
- Choi, W.-J., Kwak, J.-H., Lim, S.-S., Park, H.-J., Chang, S.X., Lee, S.-M., Arshad, M.A., Yun, S.-I., Kim, H.-Y., 2017. Synthetic fertilizer and livestock manure differently affect $\delta^{15}\text{N}$ in the agricultural landscape: a review. *Agr. Ecosyst. Environ.* 237, 1–15. <https://doi.org/10.1016/j.agee.2016.12.020>.
- Chytrý, M., Hejman, M., Hennekens, S.M., Schellberg, J., 2009. Changes in vegetation types and Ellenberg indicator values after 65 years of fertilizer application in the Rengen Grassland Experiment, Germany. *Appl. Veget. Sci.* 12 (2), 167–176.
- Cleveland, C.C., Townsend, A.R., Schmidt, S.K., 2002. Phosphorus limitation of microbial processes in moist tropical forests: evidence from short-term laboratory incubations and field studies. *Ecosystems* 5 (7), 0680–0691. <https://doi.org/10.1007/s10021-002-0020-9>.
- Cui, J., Zhang, S., Wang, X., Xu, X., Ai, C., Liang, G., Zhu, P., Zhou, W., 2022. Enzymatic stoichiometry reveals phosphorus limitation-induced changes in the soil bacterial communities and element cycling: evidence from a long-term field experiment. *Geoderma* 426. <https://doi.org/10.1016/j.geoderma.2022.116124>.
- Deng, Q., Hui, D., Dennis, S., Reddy, K.C., 2017. Responses of terrestrial ecosystem phosphorus cycling to nitrogen addition: a meta-analysis. *Glob. Ecol. Biogeogr.* 26 (6), 713–728. <https://doi.org/10.1111/geb.12576>.
- Dijkstra, F., Carrillo, Y., Pendall, E., Morgan, J., 2013. Rhizosphere priming: a nutrient perspective. *Front. Microbiol.* 4, 216.
- Dou, X., He, P., Zhu, P., Zhou, W., 2016. Soil organic carbon dynamics under long-term fertilization in a black soil of China: evidence from stable C isotopes. *Sci. Rep.* 6, 21488. <https://doi.org/10.1038/srep21488>.
- Du, E.Z., Terrer, C., Pellegrini, A.F., Ahlström, A., van Lissa, C.J., Zhao, X., Xia, N., Wu, X., Jackson, R.B., 2020. Global patterns of terrestrial nitrogen and phosphorus limitation. *Nat. Geosci.* 13 (3), 221–226. <https://doi.org/10.1038/s41561-019-0530-4>.
- Elser, J.J., Bracken, M.E., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Ngai, J.T., Seabloom, E.W., Shurin, J.B., Smith, J.E., 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol. Lett.* 10 (12), 1135–1142. <https://doi.org/10.1111/j.1461-0248.2007.01113.x>.
- Elser, J.J., Sterner, R.W., Gorokhova, E.A., Fagan, W.F., Markow, T.A., Cotner, J.B., Harrison, J.F., Hobbie, S.E., Odell, G.M., Weider, L., 2000. Biological stoichiometry from genes to ecosystems. *Ecol. Lett.* 3 (6), 540–550.
- Fan, Y., Lin, F., Yang, L., Zhong, X., Wang, M., Zhou, J., Chen, Y., Yang, Y., 2018. Decreased soil organic P fraction associated with ectomycorrhizal fungal activity to meet increased P demand under N application in a subtropical forest ecosystem. *Biol. Fertil. Soils* 54 (1), 149–161.
- Feng, J., Song, Y., Zhu, B., 2023. Ecosystem-dependent responses of soil carbon storage to phosphorus enrichment. *New Phytol.* 238 (6), 2363–2374.
- Ferrari Neto, J., Franzluebbers, A.J., Crisci, C.A.C., Rigon, J.P.G., Calonego, J.C., Rosolem, C.A., do Nascimento, C.A.C., Ribeiro, L.C., 2021. Soil carbon and nitrogen fractions and physical attributes affected by soil acidity amendments under no-till on Oxisol in Brazil. *Geoderma Reg.* 24. <https://doi.org/10.1016/j.geodrs.2020.e00347>.
- Finn, D., Page, K., Catton, K., Kienzie, M., Robertson, F., Armstrong, R., Dalal, R., 2016. Ecological stoichiometry controls the transformation and retention of plant-derived organic matter to humus in response to nitrogen fertilisation. *Soil Biol. Biochem.* 99, 117–127. <https://doi.org/10.1016/j.soilbio.2016.05.006>.
- Frank, D.A., Evans, R.D., Tracy, B.F., 2004. The role of ammonia volatilization in controlling the natural ^{15}N abundance of a grazed grassland. *Biogeochemistry* 68, 169–178.
- Freschet, G.T., Cornwell, W.K., Wardle, D.A., Elumeeva, T.G., Liu, W., Jackson, B.G., Onipchenko, V.G., Soudzilovskaia, N.A., Tao, J., Cornelissen, J.H.C., Austin, A., 2013. Linking litter decomposition of above- and below-ground organs to plant–soil feedbacks worldwide. *J. Ecol.* 101 (4), 943–952. <https://doi.org/10.1111/1365-2745.12092>.
- Godwin, C.M., Cotner, J.B., 2015. Aquatic heterotrophic bacteria have highly flexible phosphorus content and biomass stoichiometry. *ISME J.* 9 (10), 2324–2327.
- Gunina, A., Kuzyakov, Y., 2014. Pathways of litter C by formation of aggregates and SOM density fractions: Implications from ^{13}C natural abundance. *Soil Biol. Biochem.* 71, 95–104. <https://doi.org/10.1016/j.soilbio.2014.01.011>.
- Gurmesa, G.A., Hobbie, E.A., Zhang, S., Wang, A., Zhu, F., Zhu, W., Koba, K., Yoh, M., Wang, C., Zhang, Q., Fang, Y., 2022. Natural ^{15}N abundance of ammonium and nitrate in soil profiles: New insights into forest ecosystem nitrogen saturation. *Ecosphere* 13 (3). <https://doi.org/10.1002/ecs2.3998>.
- Han, G., Tang, Y., Liu, M., Van Zwieten, L., Yang, X., Yu, C., Wang, H., Song, Z., 2020. Carbon-nitrogen isotope coupling of soil organic matter in a karst region under land use change, Southwest China. *Agr. Ecosyst. Environ.* 301. <https://doi.org/10.1016/j.agee.2020.107027>.
- Hejman, M., Česková, M., Schellberg, J., Pätzold, S., 2010a. The Rengen Grassland Experiment: effect of Soil Chemical Properties on Biomass Production, Plant Species Composition and Species Richness. *Folia Geobot.* 45 (2), 125–142. <https://doi.org/10.1007/s12224-010-9062-9>.
- Hejman, M., Klauisová, M., Schellberg, J., Honsová, D., 2007. The Rengen Grassland Experiment: Plant species composition after 64 years of fertilizer application. *Agr. Ecosyst. Environ.* 122 (2), 259–266. <https://doi.org/10.1016/j.agee.2006.12.036>.
- Hejman, M., Schellberg, J., Pavlí, V., 2010b. Long-term effects of cutting frequency and liming on soil chemical properties, biomass production and plant species composition of *Lolium-cynosuretum* grassland after the cessation of fertilizer application. *Appl. Veg. Sci.* <https://doi.org/10.1111/j.1654-109X.2010.01077.x>.
- Hejman, M., Szaková, J., Schellberg, J., Tlustoš, P., 2010c. The Rengen Grassland Experiment: relationship between soil and biomass chemical properties, amount of elements applied, and their uptake. *Plant Soil* 333 (1–2), 163–179. <https://doi.org/10.1007/s11104-010-0332-3>.
- Hobbie, E.A., Hobbie, J.E., 2008. Natural abundance of ^{15}N in nitrogen-limited forests and tundra can estimate nitrogen cycling through Mycorrhizal fungi: a review. *Ecosystems* 11 (5), 815–830. <https://doi.org/10.1007/s10021-008-9159-7>.
- Hobbie, E.A., Oviatt, A.P., 2009. Controls of nitrogen isotope patterns in soil profiles. *Biogeochemistry* 95 (2–3), 355–371. <https://doi.org/10.1007/s10533-009-9328-6>.
- Hu, T.-T., Bu, D., Zhang, Y., Wang, F.-C., Li, J.-J., Zu, K.-L., Meng, M.-H., Liang, C., Fang, X.-M., 2025. Linking microbial nutrient limitation and community composition to nitrogen mineralization in bamboo forest soil with phosphorus addition. *Soil Ecol. Lett.* 7 (1), 240270.
- IUSS Working Group WRB, 2022. World Reference Base for Soil Resources. International soil classification system for naming soils and creating legends for soil maps, 4th ed. International Union of Soil Sciences (IUSS), Vienna, Austria.
- Kaiser, K., Guggenberger, G., Zech, W., 1996. Sorption of DOM and DOM fractions to forest soils. *Geoderma* 74 (3–4), 281–303.
- Kirkby, C.A., Richardson, A.E., Wade, L.J., Batten, G.D., Blanchard, C., Kirkegaard, J.A., 2013. Carbon-nutrient stoichiometry to increase soil carbon sequestration. *Soil Biol. Biochem.* 60, 77–86. <https://doi.org/10.1016/j.soilbio.2013.01.011>.
- Kriszan, M., Amelung, W., Schellberg, J., Gebbing, T., Kühbauch, W., 2009. Long-term changes of the $\delta^{15}\text{N}$ natural abundance of plants and soil in a temperate grassland. *Plant and Soil* 325 (1–2), 157–169. <https://doi.org/10.1007/s11104-009-9965-5>.
- Kriszan, M., Schellberg, J., Amelung, W., Gebbing, T., Pötsch, E.M., Kühbauch, W., 2014. Revealing N management intensity on grassland farms based on natural $\delta^{15}\text{N}$ abundance. *Agr. Ecosyst. Environ.* 184, 158–167. <https://doi.org/10.1016/j.agee.2013.11.028>.
- Li, J., Han, G., Wang, G., Liu, X., Zhang, Q., Chen, Y., Song, W., Qu, W., Chu, X., Li, P., 2022. Imbalanced nitrogen–phosphorus input alters soil organic carbon storage and mineralisation in a salt marsh. *Catena* 208. <https://doi.org/10.1016/j.catena.2021.105720>.
- Li, S., Cui, Y., Xia, Z., Zhang, X., Zhu, M., Gao, Y., An, S., Yu, W., Ma, Q., 2022. The mechanism of the dose effect of straw on soil respiration: evidence from enzymatic stoichiometry and functional genes. *Soil Biol. Biochem.* 168, 108636.
- Liu, J., Wang, C., Peng, B., Xia, Z., Jiang, P., Bai, E., 2016. Effect of nitrogen addition on the variations in the natural abundance of nitrogen isotopes of plant and soil components. *Plant and Soil* 412 (1–2), 453–464. <https://doi.org/10.1007/s11104-016-3081-0>.
- Liu, Y., Hu, C., Hu, W., Wang, L., Li, Z., Pan, J., Chen, F., 2018. Stable isotope fractionation provides information on carbon dynamics in soil aggregates subjected to different long-term fertilization practices. *Soil Tillage Res.* 177, 54–60. <https://doi.org/10.1016/j.still.2017.11.016>.
- Luo, S., Gao, Q., Wang, S., Tian, L., Zhou, Q., Li, X., Tian, C., 2020. Long-term fertilization and residue return affect soil stoichiometry characteristics and labile soil organic matter fractions. *Pedosphere* 30 (5), 703–713. [https://doi.org/10.1016/s1002-0160\(20\)60031-5](https://doi.org/10.1016/s1002-0160(20)60031-5).
- Mehnaz, K.R., Corneo, P.E., Keitel, C., Dijkstra, F.A., 2019. Carbon and phosphorus addition effects on microbial carbon use efficiency, soil organic matter priming, gross nitrogen mineralization and nitrous oxide emission from soil. *Soil Biol. Biochem.* 134, 175–186. <https://doi.org/10.1016/j.soilbio.2019.04.003>.
- Nehlich, O., 2015. The application of sulphur isotope analyses in archaeological research: a review. *Earth Sci. Rev.* 142, 1–17. <https://doi.org/10.1016/j.earscirev.2014.12.002>.

- Novák, M., Buzek, F., Harrison, A.F., Prechová, E., Jačková, I., Fottová, D., 2003. Similarity between C, N and S stable isotope profiles in European spruce forest soils: implications for the use of $\delta^{34}\text{S}$ as a tracer. *Appl. Geochem.* 18 (5), 765–779.
- Park, H.-J., Baek, N., Lim, S.-S., Jeong, Y.-J., Seo, B.-S., Kwak, J.-H., Lee, S.-M., Yun, S.-I., Kim, H.-Y., Arshad, M.A., 2023. Coupling of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to understand soil organic matter sources and C and N cycling under different land-uses and management: a review and data analysis. *Biol. Fertil. Soils* 59 (5), 487–499.
- Peñuelas, J., Janssens, I.A., Ciais, P., Obersteiner, M., Sardans, J., 2020. Anthropogenic global shifts in biospheric N and P concentrations and ratios and their impacts on biodiversity, ecosystem productivity, food security, and human health. *Glob. Chang. Biol.* 26 (4), 1962–1985.
- Peri, P.L., Ladd, B., Pepper, D.A., Bonser, S.P., Laffan, S.W., Amelung, W., 2012. Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope composition in plant and soil in Southern Patagonia's native forests. *Glob. Chang. Biol.* 18 (1), 311–321. <https://doi.org/10.1111/j.1365-2486.2011.02494.x>.
- Pötter, S., Schmitz, A., Lücke, A., Schulte, P., Obrecht, I., Zech, M., Wissel, H., Marković, S. B., Lehmkuhl, F., 2020. Middle to Late Pleistocene environments based on stable organic carbon and nitrogen isotopes of loess-palaeosol sequences from the Carpathian Basin. *Boreas* 50 (1), 184–204. <https://doi.org/10.1111/bor.12470>.
- Qaswar, M., Ahmed, W., Jing, H., Hongzhu, F., Xiaojun, S., Xianjun, J., Kailou, L., Yongmei, X., Zhongqun, H., Asghar, W., Shah, A., Zhang, H., 2019. Soil carbon (C), nitrogen (N) and phosphorus (P) stoichiometry drives phosphorus lability in paddy soil under long-term fertilization: a fractionation and path analysis study. *PLoS One* 14 (6). <https://doi.org/10.1371/journal.pone.0218195>.
- Reed, S.C., Seastedt, T.R., Mann, C.M., Suding, K.N., Townsend, A.R., Cherwin, K.L., 2007. Phosphorus fertilization stimulates nitrogen fixation and increases inorganic nitrogen concentrations in a restored prairie. *Appl. Soil Ecol.* 36 (2–3), 238–242.
- Reis, C.R., Nardoto, G.B., Rochelle, A.L., Vieira, S.A., Oliveira, R.S., 2017. Nitrogen dynamics in subtropical fringe and basin mangrove forests inferred from stable isotopes. *Oecologia* 183 (3), 841–848. <https://doi.org/10.1007/s00442-016-3789-9>.
- Rocci, K.S., Barker, K.S., Seabloom, E.W., Borer, E.T., Hobbie, S.E., Bakker, J.D., MacDougall, A.S., McCulley, R.L., Moore, J.L., Raynaud, X., Stevens, C.J., Cotrufo, M.F., 2022. Impacts of nutrient addition on soil carbon and nitrogen stoichiometry and stability in globally-distributed grasslands. *Biogeochemistry* 159 (3), 353–370. <https://doi.org/10.1007/s10533-022-00932-w>.
- Sardans, J., Janssens, I.A., Ciais, P., Obersteiner, M., Peñuelas, J., 2021. Recent advances and future research in ecological stoichiometry. *Perspect. Plant Ecol. Evol. Syst.* 50, 125611.
- Seal, R.R., 2006. Sulfur isotope geochemistry of sulfide minerals. *Rev. Mineral. Geochem.* 61 (1), 633–677. <https://doi.org/10.2138/rmg.2006.61.12>.
- Senbayram, M., Dixon, L., Goulding, K.W., Bol, R., 2008. Long-term influence of manure and mineral nitrogen applications on plant and soil ^{15}N and ^{13}C values from the Broadbalk wheat experiment. *Rapid Commun. Mass Spectrom.* 22 (11), 1735–1740. <https://doi.org/10.1002/rcm.3548>.
- Shan, Y., Huang, M., Suo, L., Zhao, X., Wu, L., 2019. Composition and variation of soil $\delta^{15}\text{N}$ stable isotope in natural ecosystems. *Catena* 183. <https://doi.org/10.1016/j.catena.2019.104236>.
- Smarda, P., Hejman, M., Brezinova, A., Horova, L., Steigerova, H., Zedek, F., Bures, P., Hejmanova, P., Schellberg, J., 2013. Effect of phosphorus availability on the selection of species with different ploidy levels and genome sizes in a long-term grassland fertilization experiment. *New Phytol.* 200 (3), 911–921. <https://doi.org/10.1111/nph.12399>.
- Sochorová, L., Jansa, J., Verbruggen, E., Hejman, M., Schellberg, J., Kiers, E.T., Johnson, N.C., 2016. Long-term agricultural management maximizing hay production can significantly reduce belowground C storage. *Agr. Ecosyst. Environ.* 220, 104–114. <https://doi.org/10.1016/j.agee.2015.12.026>.
- Spohn, M., Pötsch, E.M., Eichorst, S.A., Woebken, D., Wanek, W., Richter, A., 2016. Soil microbial carbon use efficiency and biomass turnover in a long-term fertilization experiment in a temperate grassland. *Soil Biol. Biochem.* 97, 168–175. <https://doi.org/10.1016/j.soilbio.2016.03.008>.
- Sun, Y., Wang, C., Chen, X., Liu, S., Lu, X., Chen, H.Y.H., Ruan, H., 2022. Phosphorus additions imbalance terrestrial ecosystem C:N:P stoichiometry. *Glob. Chang. Biol.* 28 (24), 7353–7365. <https://doi.org/10.1111/gcb.16417>.
- Tabatabai, M.A., Bremner, J.M., 1970. Arylsulfatase activity of soils. *Soil Sci. Soc. Am. J.* 34 (2), 225–229.
- Tang, X., Placella, S.A., Dayd, F., Bernard, L., Robin, A., Journet, E.-P., Justes, E., Hinsinger, P., 2016. Phosphorus availability and microbial community in the rhizosphere of intercropped cereal and legume along a P-fertilizer gradient. *Plant and Soil* 407 (1), 119–134.
- Vieira Fontoura, S.M., de Castro Pias, O.H., Tiecher, T., Cherubin, M.R., de Moraes, R.P., Bayer, C., 2019. Effect of gypsum rates and lime with different reactivity on soil acidity and crop grain yields in a subtropical Oxisol under no-tillage. *Soil Tillage Res.* 193, 27–41. <https://doi.org/10.1016/j.still.2019.05.005>.
- Vitousek, P.M., Porder, S., Houlton, B.Z., Chadwick, O.A., 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen–phosphorus interactions. *Ecol. Appl.* 20 (1), 5–15.
- Wang, Q., Bauke, S.L., Döring, T.F., Yin, J., Cooledge, E.C., Jones, D.L., Chadwick, D.R., Tietema, A., Bol, R., 2024. Soil pH and phosphorus availability regulate sulphur cycling in an 82-year-old fertilised grassland. *Soil Biol. Biochem.* 194. <https://doi.org/10.1016/j.soilbio.2024.109436>.
- Wang, Q., Gao, W., Bol, R., Xiao, Q., Wu, L., Zhang, W., 2022. Microbial regulation of net N mineralisation is driven by C, N, P content and stoichiometry. *Eur. J. Soil Sci.* 73 (3). <https://doi.org/10.1111/ejss.13257>.
- Wang, R., Bicharanloo, B., Hou, E., Jiang, Y., Dijkstra, F.A., 2022. Phosphorus Supply increases Nitrogen Transformation rates and Retention in Soil: a Global Meta-Analysis. *Earth's Future* 10 (3). <https://doi.org/10.1029/2021ef002479>.
- Wang, R., Peñuelas, J., Li, T., Liu, H., Wu, H., Zhang, Y., Sardans, J., Jiang, Y., 2021. Natural abundance of (^{13}C) and (^{15}N) provides evidence for plant-soil carbon and nitrogen dynamics in a N-fertilized meadow. *Ecology* 102 (6), e03348. <https://doi.org/10.1002/ecy.3348>.
- Wang, X., Li, S., Zhu, B., Homyak, P.M., Chen, G., Yao, X., Wu, D., Yang, Z., Lyu, M., Yang, Y., 2023. Long-term nitrogen deposition inhibits soil priming effects by enhancing phosphorus limitation in a subtropical forest. *Glob. Chang. Biol.* 29 (14), 4081–4093. <https://doi.org/10.1111/gcb.16718>.
- Watzka, M., Buchgraber, K., Wanek, W., 2006. Natural ^{15}N abundance of plants and soils under different management practices in a montane grassland. *Soil Biol. Biochem.* 38 (7), 1564–1576. <https://doi.org/10.1016/j.soilbio.2005.11.007>.
- Werth, M., Kuzyakov, Y., 2010. ^{13}C fractionation at the root–microorganisms–soil interface: a review and outlook for partitioning studies. *Soil Biol. Biochem.* 42 (9), 1372–1384. <https://doi.org/10.1016/j.soilbio.2010.04.009>.
- Wieder, W., Cleveland, C., Smith, W., Todd-Brown, K., 2015. Future productivity and carbon storage limited by terrestrial nutrient availability. *Nat. Geosci.* 8, 441–444. <https://doi.org/10.1038/ngeo2413>.
- Xiao, J., Dong, S., Shen, H., Zhang, R., Shi, H., He, F., Li, W., Li, X., Li, Y., Ding, C., 2025. Short-term P addition may improve the stimulating effects of N deposition on N_2O emissions in alpine grasslands on the Qinghai-Tibet Plateau. *J. Integr. Agric.* 24 (3), 900–912.
- Xu, P., Liu, Y., Zhu, J., Shi, L., Fu, Q., Chen, J., Hu, H., Huang, Q., 2020. Influence mechanisms of long-term fertilizations on the mineralization of organic matter in Ultisol. *Soil Tillage Res.* 201. <https://doi.org/10.1016/j.still.2020.104594>.
- Yang, Y.H., Fang, J.Y., Guo, D.L., Ji, C.J., Ma, W.H., 2010. Vertical patterns of soil carbon, nitrogen and carbon: nitrogen stoichiometry in Tibetan grasslands. *Biogeosci. Discuss.* 7, 1–24.
- Zechmeister-Boltenstern, S., Keiblinger, K.M., Mooshammer, M., Peñuelas, J., Richter, A., Sardans, J., Wanek, W., 2015. The application of ecological stoichiometry to plant–microbial–soil organic matter transformations. *Ecol. Monogr.* 85 (2), 133–155. <https://doi.org/10.1890/14-0777.1>.