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Biomass maturity as a key driver of green manure decomposition and soil nutrient dynamics

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Green manuring plays a vital role in enhancing soil fertility and organic matter dynamics in sustainable cropping systems, yet its effectiveness largely depends on the biochemical quality and maturity of the incorporated biomass. To elucidate the influence of crop type and development stage of buried plant biomass on soil traits, a 17-week pot experiment was conducted using safflower (*Carthamus tinctorius*), radish (*Raphanus sativus*), and white mustard (*Sinapis alba*) plants, harvested at two maturity stages: after 11 weeks (biomass A) and 12.5 weeks (biomass B) of cultivation. Biomass maturity exerted a stronger influence than crop identity on plant composition (fat, NDF, cellulose, hemicellulose), amount of residual plant biomass, and on soil properties (β-glucosidase, urease, pH). The more matured biomass (B) contained higher carbohydrates and fat contents, and despite higher recalcitrance indices, it decomposed more completely, resulting in lower residue retention. Crop-specific responses of soil traits were the most distinct at the early harvest stage: radish biomass A enhanced soil enzyme activities and total N, whereas white mustard, particularly biomass B, promoted soil P, Mg, and K availability alongside elevated N-acetyl-β-D-glucosaminidase activity. Safflower decomposed more slowly, indicating potential for longer-term carbon stabilization but limited immediate fertility gains. Overall, harvest timing emerged as a critical determinant in tailoring green manures for both nutrient cycling efficiency and soil carbon stabilization.

KEYWORDS
cover crop, decomposition, enzymes, nutrient acquisition, nutrients

Introduction

Green manuring is a widely adopted practice in sustainable and organic agriculture for maintaining soil fertility (Frøseth et al., 2014; Garcia-Franco et al., 2015), improving soil structure (Ma et al., 2024; Antosh et al., 2022), and enhancing carbon sequestration (Singh et al., 2023; Zhang et al., 2025). Green manuring particularly through the incorporation of cover crops or catch crops into soil at the green or early flowering stage (Singh et al., 2023), is a widely applied practice in organic farming. It delivers both economic benefits (Antosh et al., 2022) and ecological services that enhance soil fertility and sustainable production. By incorporating fresh plant biomass into soil, green manures provide easily decomposable organic substrates that stimulate microbial activity, nutrient mineralization, and aggregation processes. Major reported effects hitherto include improved biomass production (Antosh et al., 2022), enhancement of soil physical, chemical (Adetunji et al., 2020) and biological properties (dos Santos Cordeiro et al., 2021; Feng et al., 2021), higher soil organic matter (SOM) quality as well as C (Bogužas et al., 2015) and N contents (Liu et al., 2020), reduced nutrient leaching (Couëdel et al., 2018a), and improved nutrient availability (Su et al., 2022).

However, the benefits of green manuring depend largely on the biochemical quality and maturity of the biomass at incorporation, which govern its decomposition rate and subsequent effects on nutrient availability and soil organic carbon (SOC) stabilization. For instance, the efficacy of green manuring depends on processes following biomass incorporation through tillage (Hu et al., 2023; Li et al., 2024; Zhang et al., 2023). Incorporated biomass residues release nutrients through mineralization and transformation (Zhang et al., 2023; Sharma et al., 2000; Liu et al., 2008; Xie et al., 2022), with release dynamics influenced by residue stoichiometry, particularly the C:N ratio (Ranaivoson et al., 2022). These processes are accompanied by enhanced enzymatic activity (Xu W. et al., 2023; Piotrowska-Dlugosz and Wilczewski, 2020; Xu J. et al., 2023), which further improves nutrient availability (Xu W. et al., 2023; Solangi et al., 2019; Dong et al., 2021) and short-term use efficiency (Muhammad et al., 2022; Wang et al., 2025). Consequently, green manuring often leads to higher crop yields (Zhang et al., 2023; Sharma et al., 2000; Xie et al., 2022; Gautam et al., 2021).

The decomposition dynamics of green manures are closely linked to their chemical composition, including the proportions of carbohydrates, lignin, cellulose, hemicellulose, and fat (Sharma et al., 2000; Liu et al., 2008). A key factor shaping these outcomes is the maturity stage of the incorporated biomass (Sandhu et al., 2022). Younger residues typically contain easily degradable lipids, proteins, and non-structural carbohydrates (Feng, 2022; Jenkinson, 1965; Gunnarsson et al., 2008), whereas more mature residues are enriched in cellulose, hemicellulose (Henriksen and Breland, 1999), and lignin (Jensen et al., 2005; Moore et al., 1999), conferring stability and recalcitrance (Hadas et al., 2004). Therefore, identifying the optimal harvest stage that balances nutrient release and carbon stabilization remains a major challenge in optimizing green manuring systems. Despite the recognized importance of maturation stage at harvesting for biomass quality, most studies on green manures have emphasized species selection or management frequency (Singh et al., 2023; Antosh et al., 2022; Zhang et al., 2023; Sharma et al., 2000; Liu et al., 2008; Xie et al., 2022), while the influence of biomass maturity, particularly in short-duration crops remains poorly quantified. A deeper understanding of how subtle differences in crop maturity alter biochemical composition, decomposition patterns, and soil responses is essential to refine green manure management for both short-term fertility improvement and long-term carbon stabilization.

To address this research gap, we conducted a 17-week pot experiment using three green manure crops—safflower (*Carthamus tinctorius*), radish (*Raphanus sativus*), and white mustard (*Sinapis alba*), harvested at two maturity stages (11 and 12.5 weeks). The objectives were to (i) evaluate how crop type and maturity stage affect biomass composition and decomposition, (ii) assess their impacts on soil enzyme activities and nutrient availability, and (iii) identify the optimal maturity stage for maximizing both soil fertility and carbon stabilization potential of green manures.

Materials and methods

The crop biomass acquirement

Incorporated plants were applied as aboveground biomass: radish (*Raphanus sativus* L.), safflower (*Carthamus tinctorius* L.), and white mustard (*Sinapis alba* L.) were harvested from fields near Nová Ves, Brno (49°06′25.7″ N, 16°18′15.3″ E) after 77 days (11 weeks; biomass A) or 87 days (12.5 weeks; biomass B; Table 1). Plants were cut at ground level, oven-dried at 60 °C to constant weight, analysed (details in the section *Plant and soil analyses*) and stored.

Soil preparation and experimental design

The experiment was conducted at Agricultural Research, Ltd. (Troubsko, Czech Republic; 49°17′49.8″ N, 16°49′13.0″ E). Arable topsoil (0–15 cm) was collected from fields managed conventionally in a cereal–rapeseed–pea rotation. The site is moderately warm and dry (mean annual temperature 9.0 °C, precipitation 526 mm). Geologically, it is underlain by loess/loess loam of the Bohemian Massif. The soil was classified as a Haplic Luvisol (WRB) with silty clay loam texture (USDA). The primery chemical properties were: total C 14.0 $g{\cdot}kg^{-1}$, total N 1.60 $g{\cdot}kg^{-1}$, P 0.10 $g{\cdot}kg^{-1}$, S 0.15 $g{\cdot}kg^{-1}$, Ca 3.26 $g{\cdot}kg^{-1}$, Mg 0.24 $g{\cdot}kg^{-1}$, K 0.23 $g{\cdot}kg^{-1}$; pH ($CaCl_2$) 7.3. Soil was homogenized and sieved (2 mm, Retsch GmbH, Germany).

The trial was conducted in rectangular boxes (57 × 37 cm; 10 kg soil each). Two semipermeable polyamide mesh bags (Uhelon 130 T, 42 nm pore size, SILK and PROGRESS, Czech Republic), each containing 20 g of dry biomass (equivalent to 2 $g{\cdot}kg^{-1}$ soil), were placed in each box and covered with 2 cm soil layer (Supplementary Figure S1). All treatments (Table 2) were arranged in triplicate (n = 3).

The experiment was conducted from 16 February to 16 June 2022 (120 days). Environmental conditions were maintained at 9/16 °C (night/day), and each box was watered weekly with 1.5 L of distilled water to simulate alternating dry and moist periods. Urea was applied at a rate of 3.44 g per box on days 15 and 35 after incubation began. This meant than soil was fertilized with urea (46% N, AGRO CS a.s., Czech Republic) at dose of 0.688 g $N{\cdot}kg^{-1}$ (150 kg $N{\cdot}ha^{-1}$), applied in two splits.

During sampling at the end of the trial, soil was gently removed, mesh bags collected, and soil sampled from the 0–2 mm layer beneath each bag. Residual biomass (RB; $g{\cdot}bag^{-1}$) was oven-dried (65 °C) and weighed.

Plant and soil analyses

Plant biomass

Fibre fractions were determined as acid-detergent fibre (ADF) and neutral-detergent fibre (NDF) (ANKOM, 2006), using ANKOM

TABLE 1 The cover crops used in the pot trial.

#	acronym	Intercrop species	term of sowing	term of harvest	type
1	Rad1	Radish (*Raphanus sativus* L.)	16.8. 2021	1.11.2021	A
2	SafA	Safflower (*Carthamus tinctorius* L.)	16.8. 2021	1.11.2021	A
3	Wmu1	white mustard (*Sinapis alba* L.)	16.8. 2021	1.11.2021	A
4	RadB	Radish (*Raphanus sativus* L.)	16.8. 2021	11.11.2021	B
5	SafB	Safflower (*Carthamus tinctorius* L.)	16.8. 2021	11.11.2021	B
6	Wmu2	white mustard (*Sinapis alba* L.)	16.8. 2021	11.11.2021	B

TABLE 2 The experimental variants (their acronyms) according to buried crops and WA treatment.

Buried intercrop	Variant	Plant biomass per box	Urea per box
Control	Cont	-	-
Radish (*Raphanus sativus* L.)	RadA	20 g	6.88 g
Safflower (*Carthamus tinctorius* L.)	SafA	20 g	6.88 g
white mustard (*Sinapis alba* L.)	WmuA	20 g	6.88 g
Radish (*Raphanus sativus* L.)	RadB	20 g	6.88 g
Safflower (*Carthamus tinctorius* L.)	SafB	20 g	6.88 g
white mustard (*Sinapis alba* L.)	WmuB	20 g	6.88 g

220 fibre analyser (ANKOM Technology, USA). Acid-detergent lignin (ADL) was measured according to ISO 13906:2008 (ISO_13906, 2008). Hemicellulose was calculated as NDF − ADF and cellulose as ADF − ADL (Rinne et al., 1997). Crude protein was determined by the Kjeldahl method using Kjeltec™ 2300 Analyzer (FOSS, Denmark) (AOAC, 2023) and calculated with equation: protein content = total N × 6.25. Crude fibre was determined by two-step hydrolysis (H_2SO_4 followed by KOH) with ash correction. Reducing sugars were measured by the Luff–Schoorl method (Pomeranz and Meloan, 1994; Marrubini et al., 2017). Ash was determined according to (ISO_6865, 2000). Fat content was determined gravimetrically using the Soxhlet extraction with petroleum ether (Garcı´a-Ayuso and Luque de Castro, 1999).

Soil

Fresh soil was sieved (2 mm; Retsch, Germany) and soil subsamples were processed differently according to which analyses they were used for. Air-dried subsamples were analysed for pH($CaCl_2$) ISO 10390:2005 (ISO_10390, 2005), oxidizable carbon (Cox), determined by sulfochromic oxidation ISO_14235 (1998), and total nitrogen (TN) ISO 13878:1998, measured by dry combustion (ISO_13878, 1998), using a Vario Macro Cube (Elementar Analysensysteme GmbH, Langenselbold, Germany). The Cox_N ratio was calculated from Cox and TN. Soil phosphorus (P), magnesium (Mg) and potassium (K) were quantified in the soil extract, prepared according to the method based on Mehlich III reagent (Mehlich, 2008), using the Agilent 7700x ICP-MS featuring the Octopole Reaction System (Agilent Technologies, Japan).

Soil enzyme assays

Sieved soil (<2 mm) was used fresh or stored at 4 °C for dehydrogenase activity (DHA) determined by the TTC method (Doi and Ranamukhaarachchi, 2009) expressed as µg $\text{TPF}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$. Freeze-dried soil was analysed for β-glucosidase (GLU; C-mineralising), urease (Ure; N-mineralising), N-acetyl-β-D-glucosaminidase (NAG; N-mineralising), phosphatase (Phos; P-mineralising), and arylsulfatase (ARS; S-mineralising) following (ISO_20130, 2018). Activities were measured spectrophotometrically at λ = 405 nm (PNP assays) or 650 nm (urease), expressed as nmol $\text{PNP}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ (enzymes) or nmol $\text{NH}_3\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ (urease).

Stoichiometric metrics

Nutrient acquisition ratios were calculated according to Cui et al. (2022), using the equations for C (Equation 1) and N (Equation 2).

$$\text{C acquisition ratio} = \ln(\text{DHA+GLU}) \,/\, \ln(\text{DHA+GLU} + \text{NAG} + \text{Ure}) \tag{1}$$

$$\text{N acquisition ratio} = \ln(\text{NAG+Ure}) \,/\, \ln(\text{NAG+Ure} + \text{Phos}) \tag{2}$$

Microbial resource limitation was evaluated by vector analysis according to Moorhead et al. (2016), using the Equations 3, 4:

$$Vector\ length = \sqrt{\left(\frac{\ln(DHA+GLU)}{\ln(Phos)}\right)^2 + \left(\frac{\ln(DHA+GLU)}{\ln(NAG+Ure)}\right)^2} \tag{3}$$

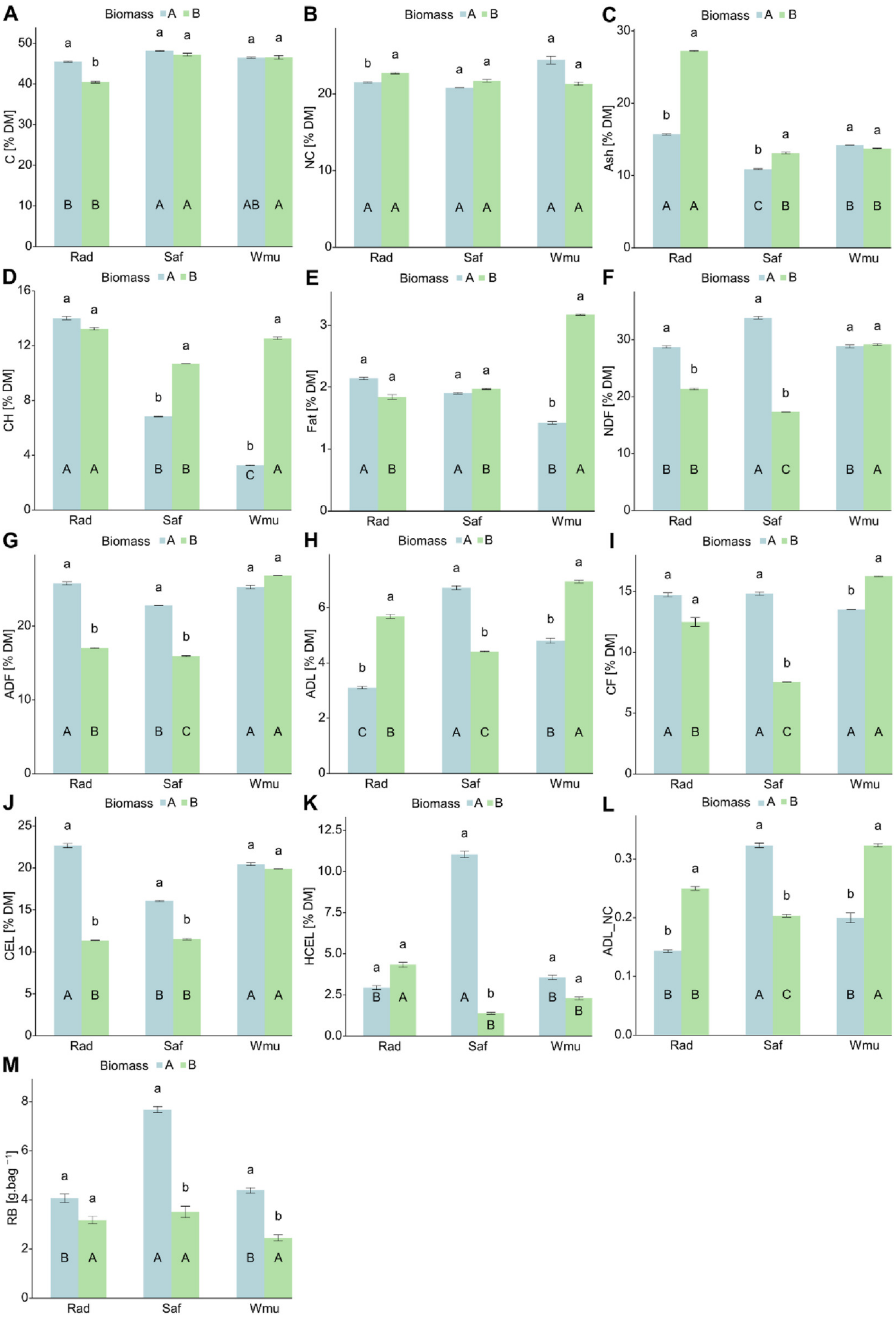

FIGURE 1
Initial properties of plant biomass **(A–H)**. Properties: carbon content (C - chart **(A)**), content of nitrous compounds (NC - chart **(B)**), Ash (chart **(C)**), carbohydrate content (CH - chart **(D)**), fat (chart **(E)**), neutral detergent fiber (NDF - chart **(F)**), acid detergent fiber (ADF - chart **(G)**), acid detergent lignine (ADL - chart **(H)**), crude fiber (CF - chart **(I)**), cellulose content (CEL - chart **(J)**), hemicellulose content (HCEL - chart **(K)**), ADL to NC ratio (ADL_NC - chart **(L)**), residual biomass (RB - chart **(M)**); displayed are means (n = 3) ± standard error of mean (SEM; error bars); lowercase letters express differences among two types of biomass for a single crop (in regard to the date of harvest), uppercase letters express differences among variants (crop type) within each group with simultaneously harvest biomass; results based on Tukey's HSD post-hoc test calculated at a significance level $p \leq 0.05$. Rad = radish; Saf = safflower, Wmu = white mustard.

$$Vector\,angle\,(rad) = ARCTG2\frac{\ln(DHA+GLU)}{\ln(Phos)};\frac{\ln(DHA+GLU)}{\ln(NAG+Ure)} \quad (4)$$

where ARCTG2 denotes the arctangent.

Statistic analyses

All data (plant biomass and quality traits, soil chemical and biological parameters) were analysed in R v4.3.1 (R_Core_Team, 2023). Pearson's correlations were used to characterise relationships, interpreted as moderate (0.5–0.7), high (0.7–0.9), or very high (>0.9) (Hinkle et al., 2003). Figures were created using ggplot2 0.5–0.7 = moderate, 0.7–0.9 = high, and >0.9 = very high correlation. Figures were created using *ggplot2* package (Wickham, 2016).

One-way and two-way ANOVA with Type I sums of squares were applied at α = 0.05 (Zar, 1984), using the *FactoMineR* (Lê et al., 2008) and *Factoextra* (Kassambara and Mundt, 2020) packages. Tukey's HSD (α = 0.05) was used for *post hoc* comparisons. Results are presented as standard error of the mean (±SEM) (Faraway, 2014).

Model assumptions were tested at α = 0.05. Normality of residuals was evaluated with Kolmogorov–Smirnov and Anderson–Darling tests (nortest package). Homoscedasticity was assessed by Bartlett's and Fligner–Killeen tests. Diagnostic plots included residuals vs. fitted values, Q–Q plots, Cook's distance, and leverage plots (Faraway, 2014; Pekár and Brabec, 2016). Distributional shape was further tested using D'Agostino's K-squared (skewness) and Anscombe–Glynn (kurtosis) tests (moments package (Komsta and Novomestky, 2022).

Results

Biodegradation and properties of plant biomass

Chemical and nutritional traits of plant biomass, which determine decomposition rates, varied significantly with both crop species and harvest time (one-way ANOVA; partial eta-squared; Supplementary Table S1; Figures 1A–H).

Carbon content (C, % DM) was lower in radish compared to safflower and white mustard, and also differed between RadA and RadB (Figure 1A). Nitrogen content (N, % DM) did not vary among crop species but was higher in RadB than in RadA (Figure 1B). Ash content (% DM) was highest in radish for both harvests; however, later harvest increased Ash in both radish and safflower (Figure 1C).

Carbohydrates (CH, % DM) in biomass A showed a decreasing trend from radish (highest) to white mustard (lowest), whereas in biomass B radish and white mustard were comparable, and both SafB and WmuB increased with ripening time (Figure 1D). Fat (% DM) content showed the strongest contrast between harvest stages in white mustard, with the lowest values in WmuA and the highest in WmuB (Figure 1E).

Neutral detergent fibre (NDF) and acid detergent fibre (ADF, % DM) decreased in radish and safflower biomass B compared to biomass A. In contrast, WmuB had significantly higher NDF, ADF, and acid detergent lignin (ADL, % DM) than the other crops at the later harvest (Figures 1F–H). ADL in biomass A was highest in SafA but declined in SafB, while RadB and WmuB were higher than RadA and WmuA (Figure 1H).

Similar to NDF, ADF, and ADL, crude fibre (CF) and cellulose (CEL, % DM) values were highest in WmuB (Figures 1I,J). In biomass A, CEL was reduced in SafA. For safflower, CF and CEL decreased from SafA to SafB, while hemicellulose (HCEL, % DM) showed the opposite trend: highest in SafA but reduced in SafB compared to the other crops (Figure 1K).

The ratio of ADL to nitrogenous compounds (ADL_NC), an indicator of digestibility, closely followed ADL patterns. Values increased from biomass A to biomass B in radish and white mustard, but decreased in safflower (SafB < SafA; Figure 1L).

Residual biomass (RB, % DM) was significantly higher in SafA than in the other two crops, indicating slower decomposition of safflower. However, RB values were comparable across crops in biomass B (Figure 1M).

Soil properties

Soil pH did not differ among variants with various incorporated crops, neither in earlier (biomass A) or later maturity stage (biomass B). However, biomass B amendments generally lowered pH compared with unamended soil (Figure 2C). In particular, RadB- and WmuB-amended soils had lower pH than RadA and WmuA, respectively (Figure 2A).

Soil contents of oxidizable organic carbon (Cox), total carbon (TC), total nitrogen (TN), and their ratio (Cox/TN) were unchanged across crops within both maturity stages (Figures 3A–I). By contrast, total phosphorus (P) and potassium (K) increased in soils amended with biomass A, showing a trend from lower values in RadA to higher values in WmuA, all exceeding the unamended control (Figures 4B,H). Biomass B amendments also elevated P, K, and Mg contents relative to the control, except for P in SafB and Mg in RadB (Figures 4C,F,I). Within each maturity stage, Mg did not differ among crops (Figure 4D). P and K were highest in WmuA soils, while P was highest in WmuB soils, compared with RadA (P, K) and SafB (P) (Figures 4A,G).

Enzyme activities responded variably to biomass incorporation. β-glucosidase (GLU) activity increased in RadA- and SafA-amended soils compared with the control (Figure 5B), but showed no differences among crop treatments within each maturity stage (Figure 5A). Phosphatase (Phos) activity was higher in RadA and SafB than in the control (Figures 5E,F). Across maturity stages, biomass A amendments generally enhanced GLU (all crops) and Phos (radish and white mustard) compared to biomass B (Figure 5A).

Urease (Ure) activity increased in SafA but decreased in RadB compared with the control (Figures 6B,C). Differences among crops were also evident: WmuA and RadB had lower Ure activity than the other two crops at the same maturity stage (Figure 6A). N-acetyl-β-D-glucosaminidase (NAG) and arylsulfatase (ARS) increased in RadA relative to the control (Figures 6E,H). Biomass B amendments further raised NAG in WmuB and ARS in RadB and SafB (Figures 6F,I). Among crop comparisons, WmuA decreased ARS relative to RadA, whereas WmuB increased NAG compared to RadB and SafB (Figures 6D,G).

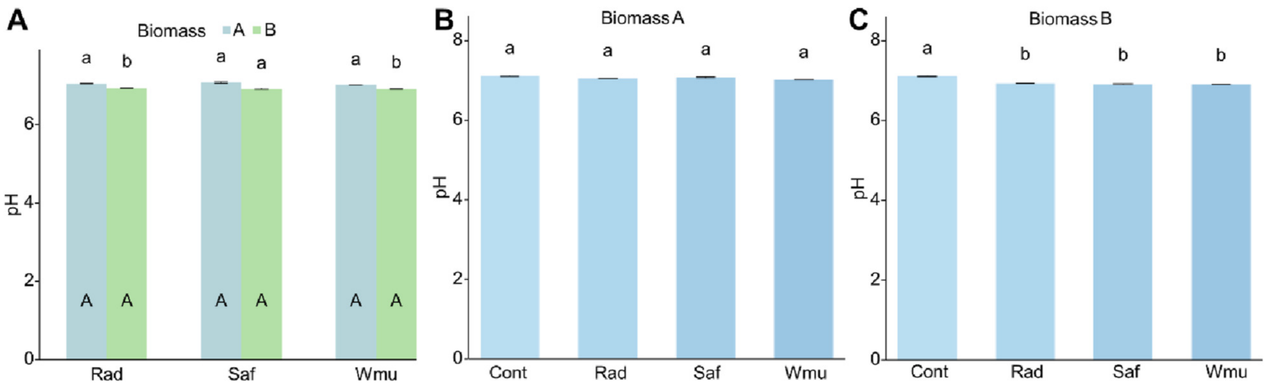

FIGURE 2
Soil pH at the end of trial. Displayed are means (n = 3) ± standard error of mean (SEM; error bars); chart **(A)** - lowercase letters expressed differences among variants (crop type) within a group of variants with equal type of biomass [either **(A,B)**]; charts **(B,C)** - lowercase letters express differences among variants with an equal crop (differing in the date of harvest), uppercase letters express differences among variants discriminated according to the crop type; results based on Tukey's HSD post-hoc test calculated at a significance level $p \leq 0.05$. Rad = radish; Saf = safflower, Wmu = white mustard.

Enzymatic stoichiometry also shifted with crop type and maturity. The soil carbon acquisition ratio (Cacq.) and vector length (C limitation index) remained unchanged with biomass A but increased under RadB compared with other crops (Figures 7C,I). Within biomass A, WmuA showed higher Cacq. and vector length than SafA, while RadB had the highest values among biomass B treatments (Figures 7A,G).

Nitrogen acquisition ratio (Nacq.) and vector angle (N limitation index) showed opposite patterns. RadB decreased Nacq. but increased vector angle relative to the control (Figures 7F,L). Within biomass A, WmuA had lower Nacq. and higher angle than SafA. Conversely, WmuB had the highest Nacq. and lowest angle among biomass B treatments. Across crops, Nacq. declined while vector angle increased with longer maturation (biomass A vs. B; Figures 7D,J).

The relations among plant and soil properties

Pearson's correlation analysis (Supplementary Figure S2A revealed several significant relationships. Plant C content correlated negatively with Ash content ($p \leq 0.001$, $r = -0.89$) and with the soil Vector index ($p \leq 0.01$, $r = -0.65$), but positively with soil urease (Ure) activity and N acquisition ratio (Nacq.) ($p \leq 0.001$, $r = 0.74$ and 0.71, respectively). Conversely, Ash content correlated negatively with Ure and Nacq. ($p \leq 0.001$, $r = -0.80$ and -0.73). Acid detergent fibre (ADF) correlated positively with Nacq. ($p \leq 0.01$, $r = 0.63$), while neutral detergent fibre (NDF) and residual biomass (RB) correlated positively with soil pH ($p \leq 0.01$, $r = 0.61$; $p \leq 0.001$, $r = 0.72$). Acid detergent lignin (ADL) correlated negatively with β-glucosidase (GLU; $p \leq 0.01$, $r = -0.60$). Residual biomass correlated positively with hemicellulose (HCEL; $p \leq 0.001$, $r = 0.83$). Among soil parameters, phosphatase (Phos) correlated negatively with P, K, and Mg contents ($p \leq 0.01$, $r = -0.65, -0.70, -0.73$).

When analysed separately for biomass A (Supplementary Figure S2B), RB correlated positively with NDF, HCEL, and ADL ($p \leq 0.001$, $r = 0.87, 0.93, 0.85$), and negatively with ADF, cellulose (CEL), and Ash ($p \leq 0.001$, $r = -0.81, -0.88, -0.95$).

Discussion

Crop-specific quality and maturity stage impacts plant biomass properties

The results of residual biomass quantification, soil trait and plant quality determination indicated that both crop choice and, more decisively, harvest timing are critical determinants of biomass quality and subsequent soil responses. Early-harvested biomass provides more labile substrates that promote larger decomposition, whereas later-harvested biomass, particularly of white mustard, is more resistant and may contribute to long-term soil organic matter stabilization. Specifically, residual biomass (RB; Figure 1M) indicated more extensive biodegradation of all crops (77%–87% mass loss), except safflower biomass A (SafA), which decomposed less (62% loss). This lower degradability of SafA was associated with higher NDF, ADL, hemicellulose, and ADL:NC ratios, and reduced cellulose and Ash. High lignin content (Jensen et al., 2005), hemicellulose (Henriksen and Breland, 1999), and elevated ADL:nitrogen ratios (Moore et al., 1999) are generally linked with structural stability and decomposition resistance. Conversely, high protein, fat, and soluble carbohydrates support easier degradation (Heal et al., 1997; Aditya and Wolfram, 2020).

Interestingly, the most degraded biomasses such as RadA and WmuB did not show higher nitrogenous compound contents, challenging the assumption that N-rich biomass always decomposes easily. Instead, both were rich in fat, fibre, and cellulose. Although these parameters cause moderately recalcitrant decay (Henriksen and Breland, 1999; Zoghlami and Paës, 2019), cruciferous crops (radish, white mustard) tended to degrade more easily, consistent with previous observations (Kucerik et al., 2024). By contrast, safflower decomposed less easily, despite relatively low ADL and fibre fractions in SafB, highlighting inconsistency with previous reports of safflower green manure contributing substantially to soil C (Kucerik et al., 2024).

As aforementioned, decomposition was shaped by both crop type and maturity stage (two-way ANOVA, Supplementary Table

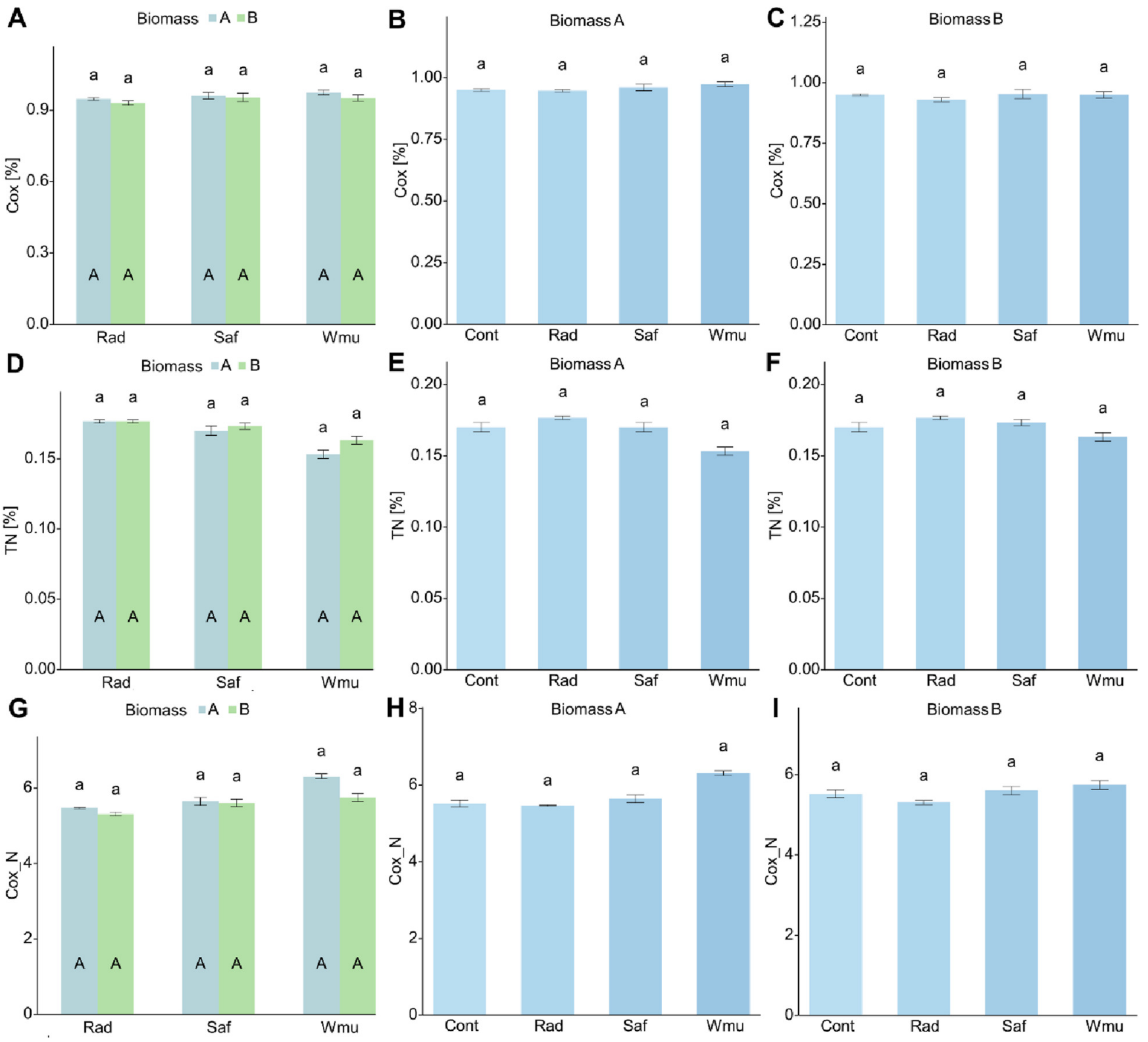

FIGURE 3
Soil oxidizable carbon (Cox - charts **(A-C)**), total nitrogen (TN - charts **(D-F)**), and Cox_N (charts **(G-I)**) at the end of trial. Displayed are means (n = 3) ± standard error of mean (SEM; error bars); charts **(A,D,G)** - lowercase letters expressed differences among variants (crop type) within a group of variants with equal type of biomass [either **(A,B)**]; charts **(B,C,E,F,H,I)** - lowercase letters express differences among variants with an equal crop (differing in the date of harvest), uppercase letters express differences among variants discriminated according to the crop type; results based on Tukey's HSD post-hoc test calculated at a significance level $p \leq 0.05$. Rad = radish; Saf = safflower, Wmu = white mustard.

S1). In some traits (e.g., fat, NDF, hemicellulose), harvest stage had a stronger effect than crop identity. Correlation analysis confirmed that RB was positively associated with hemicellulose and NDF, and negatively with lignin, cellulose, and Ash (Supplementary Figure S2). This suggests that decomposition depends not only on the abundance of resistant polymers, but also on the specific balance between moderately degradable (cellulose, hemicellulose) and resistant (lignin, Ash) compounds.

These results indicate a fundamental difference between crucifers and safflower: cruciferous residues appear more degradable despite moderately recalcitrant fibre fractions, possibly due to their biochemical composition (e.g., glucosinolates and associated breakdown products), while safflower shows unexpectedly slow decomposition even at lower ADL contents. Such a decoupling between bulk ADL and decay rate is consistent with evidence that lignin chemistry and phenolic cross-linking (e.g., ferulate-mediated linkages) can physically and chemically protect labile carbohydrates, thereby constraining decomposition independent of ADL concentration (Hatfield et al., 2017; Talbot et al., 2012).

Therefore, the results imply that cruciferous crops appear more degradable, making them better suited for short-term nutrient release, while safflower may stabilize soil C. A combination of cruciferous residues with safflower could therefore balance rapid nutrient turnover with longer-term carbon sequestration, a strategy that could be agronomically exploited for soil fertility management.

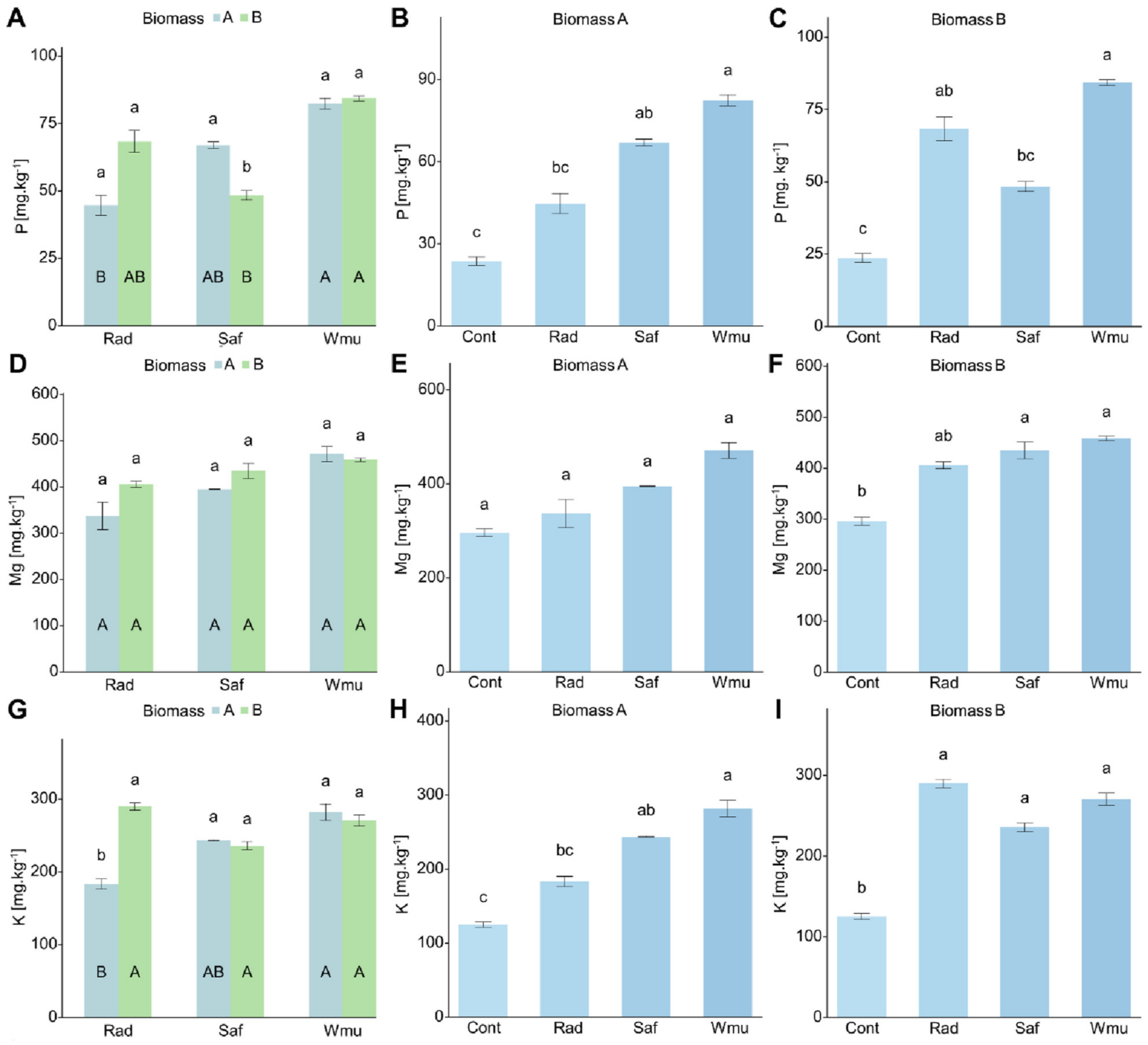

FIGURE 4
Soil total phosphorus (P - charts **(A-C)**), magnesium (Mg - charts **(D-F)**), and potassium (K - charts **(G-I)**) at the end of trial. Displayed are means (n = 3) ± standard error of mean (SEM; error bars); charts **(A,D,G)** - lowercase letters expressed differences among variants (crop type) within a group of variants with equal type of biomass [either **(A,B)**]; charts **(B,C,E,F,H,I)** - lowercase letters express differences among variants with an equal crop (differing in the date of harvest), uppercase letters express differences among variants discriminated according to the crop type; results based on Tukey's HSD post-hoc test calculated at a significance level p ≤ 0.05. Rad = radish; Saf = safflower, Wmu = white mustard.

Impact on soil pH

Previous studies often report that green manuring raises soil pH (Xu W. et al., 2023; Wang et al., 2024). In this experiment, crop type did not significantly affect pH, but biomass B amendments slightly decreased pH compared to the control. This acidifying effect was linked to altered Mg:K ratios: unamended soil had Mg:K ≈ 2.4, while biomass B-amended soils ranged from 1.4 to 1.8. Such shifts in cation ratios can influence soil buffering capacity and mineral availability (Jalali, 2008).

Although the magnitude of pH change was small, it appeared that even minor acidification may accumulate under repeated applications of green manures, especially when combined with shifts in Mg:K balance. This could alter root nutrient uptake and rhizosphere buffering, implying that crop maturity selection could serve as a management tool to fine-tune soil reaction for target crops.

Impacts on soil carbon, mineral content and transformation

Stoichiometric indicators revealed that radish at later harvest (RadB) intensified microbial C limitation (higher C acquisition and vector length) but reduced N acquisition, accompanied by greater N limitation (higher vector angle). In contrast, white mustard at later harvest (WmuB) promoted microbial N acquisition and alleviated N limitation. These findings suggest that the maturity stage of

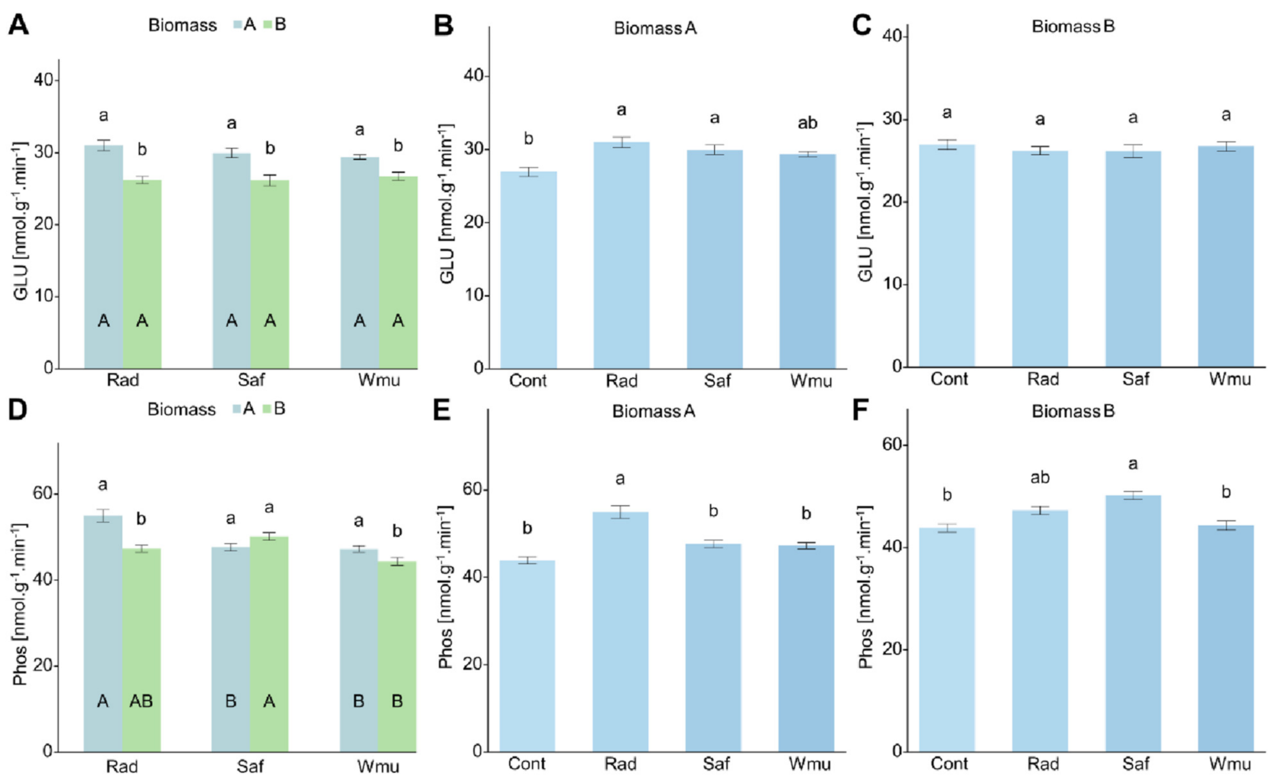

FIGURE 5
Soil enzymes mineralizing carbon and phosphorus at the end of trial. Displayed are means (n = 3) ± standard error of mean (SEM; error bars) of β-glucosidase (GLU - charts **(A-C)**) and phosphatase (Phos - charts **(D-F)**); charts **(A,D)** - lowercase letters expressed differences among variants (crop type) within a group of variants with equal type of biomass [either **(A,B)**]; charts **(B,C,E,F)** - lowercase letters express differences among variants with an equal crop (differing in the date of harvest), uppercase letters express differences among variants discriminated according to the crop type; results based on Tukey's HSD post-hoc test calculated at a significance level $p \le 0.05$. Rad = radish; Saf = safflower, Wmu = white mustard.

incorporated biomass is a decisive factor also in regulating soil nutrient availability and microbial resource allocation. Early incorporation enhances labile nutrient cycling, while later incorporation, particularly of white mustard, supports more balanced nutrient acquisition and may favour longer-term soil fertility.

In general, green manures are linked to higher soil C (Patra et al., 2023), however, in this study, no significant changes in oxidizable C were detected. Radish (lower plant C) degraded faster, whereas safflower (higher plant C) decomposed slowly, reducing its contribution to soil C enrichment. This illustrates a trade-off: C-rich biomass may not enrich soil if its decomposition is too slow to release labile substrates (Tamura et al., 2017; Gleixner et al., 2001). In general, the length of the experiment was too short to consider markable changes in total C contents that would be likely observed in similar medium- to long-termed trial.

Enzyme responses supported this view. β-glucosidase (GLU) activity was unchanged under biomass B but increased under RadA and SafA, coinciding with higher cellulose + hemicellulose contents and carbohydrate levels. Higher hemicellulose:ADL ratios in early harvests (A) compared to later harvests (B) likely explained these effects (Liu et al., 2025). Thus, enzyme activity was driven more by substrate quality than crop type alone.

C acquisition (Cacq.) and microbial C limitation (Vector length) responded only to biomass B, and most strongly in RadB. This biomass had high HCEL:ADL and Ash but low plant C and C:N, promoting greater microbial demand and C turnover. In contrast, soil P, K, and Mg generally increased with biomass amendments, consistent with other reports for P (Zhang et al., 2023; Kataria et al., 2024; Khan et al., 2025), potassium and magnesium (Solangi et al., 2019; Kucerik et al., 2024; Ma et al., 2021).

Interestingly, higher phosphatase activity (Phos) occurred in RadA and SafB, both associated with lower soil P content. This suggests microbial upregulation of P-mining under limitation, corroborated by higher vector angles (>45°) aligning with the notion about mutual relation between reduced availability of P and induced Phos (functioning also vice-versa (Bargaz et al., 2012; Janes-Bassett et al., 2022)).

Altogether, these results show that the maturity stage determines whether soil C inputs stimulate microbial activity (labile C cycling, possible priming of SOM) or contribute to persistent organic matter pools (slow decomposition, reduced turnover). The consistent link between stoichiometric indices and enzyme assays highlights that integrating these approaches is essential to reveal "hidden" nutrient limitations and microbial allocation trade-offs.

Impacts on soil enzymes and related elements

Correlation analysis confirmed strong links between plant biomass traits and soil enzyme dynamics. High C content in plant material was

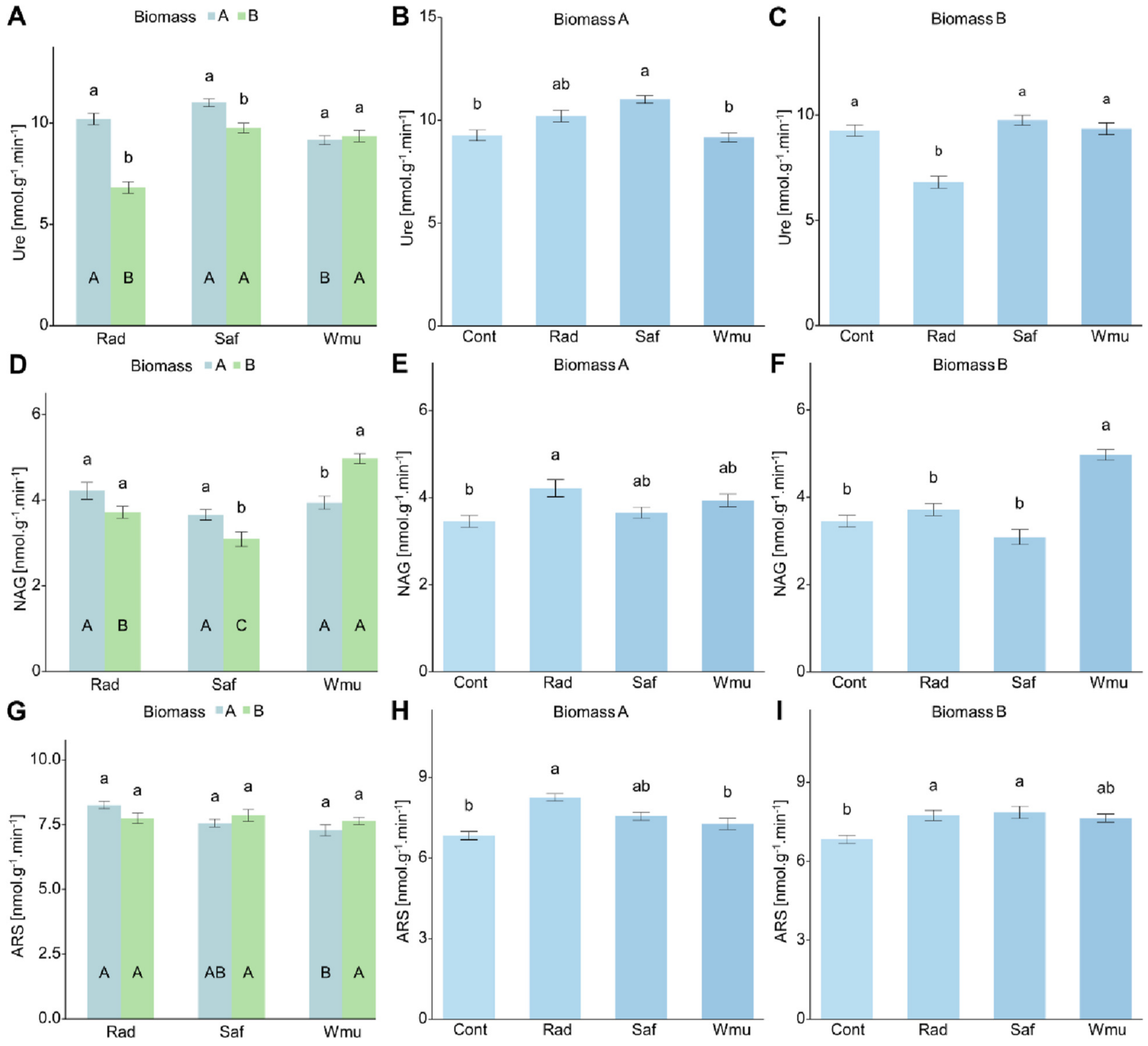

FIGURE 6
Soil enzymes mineralizing nitrogen and sulphur at the end of trial. Displayed are means (n = 3) ± standard error of mean (SEM; error bars) of urease (Ure - charts **(A-C)**), N-acetyl-β-D-glucosaminidase (NAG - charts **(D-F)**), and arylsulfatase (ARS - charts **(G-I)**) charts **(A,D,G)** - lowercase letters expressed differences among variants (crop type) within a group of variants with equal type of biomass [either **(A,B)**]; charts **(B,C,E,F,H,I)** - lowercase letters express differences among variants with an equal crop (differing in the date of harvest), uppercase letters express differences among variants discriminated according to the crop type; results based on Tukey's HSD post-hoc test calculated at a significance level $p \leq 0.05$. Rad = radish; Saf = safflower, Wmu = white mustard.

associated with increased urease activity and microbial N acquisition, while high Ash content showed the opposite trend, indicating that mineral-rich biomass may partially suppress N cycling. Fibre quality traits also played a key role: hemicellulose and lignin were strongly associated with residual biomass, suggesting that structural compounds drive decomposition resistance, while lignin negatively correlated with β-glucosidase, reflecting reduced microbial C acquisition from recalcitrant residues. Among soil properties, higher P, K, and Mg contents were linked with lower phosphatase activity, implying nutrient feedback regulation.

Contrary to some studies (Lyu et al., 2023; Wang et al., 2025), biomass incorporation did not significantly change soil TN or C:N ratios in this study. However, enzyme activities indicated shifts in N cycling. Urease activity increased under SafA, while WmuA and RadB showed reduced values compared to other treatments. This suggests that safflower may sustain N mineralization despite its slower decomposition.

N-acetyl-β-D-glucosaminidase (NAG), linked to fungal chitin turnover, increased in RadA and WmuB relative to the control. Crucifer biomass maturity negatively affected NAG, as seen in lower activity under SafB versus SafA, aligning with reports that later maturity reduces protein degradation (Kohn and Allen, 1995; Kejun et al., 2011). Arylsulfatase (ARS) also increased with biomass amendments, consistent with earlier findings (Tejada et al., 2008; Elfstrand et al., 2007). However, WmuA showed the lowest ARS, despite white mustard being rich in S-containing glucosinolates

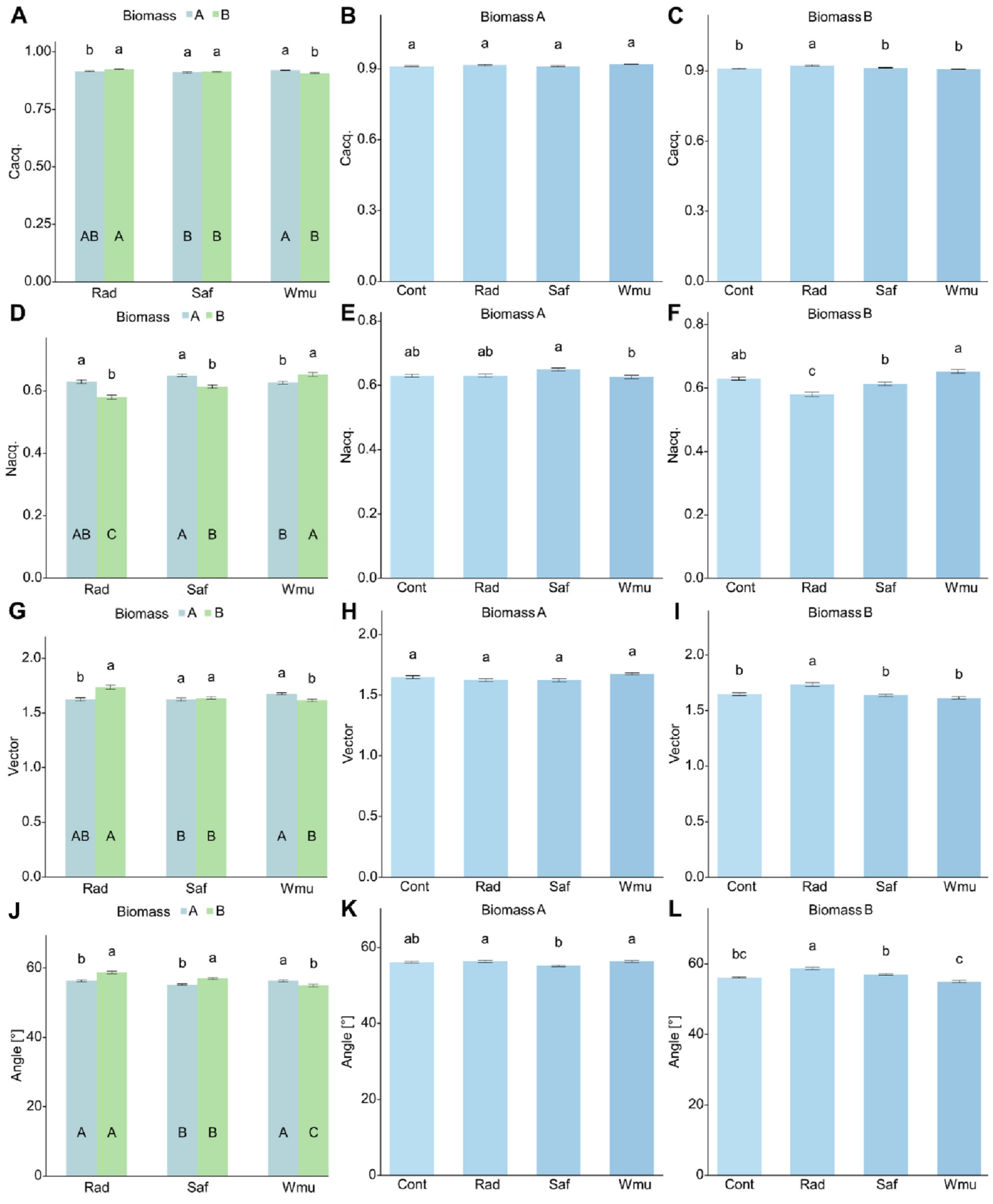

FIGURE 7
Soil indicators of nutrient acquisition and limitation at the end of trial. Displayed are means (n = 3) ± standard error of mean (SEM; error bars) of carbon acquisition (Cacq. - charts **(A-C)**) and limitation (Vector - charts **(G-I)**), and nitrogen acquisition (Nacq. - charts **(D-F)**) and limitation (Angle - charts **(J-L)**); charts **(A,D,G)** - lowercase letters expressed differences among variants (crop type) within a group of variants with equal type of biomass [either **(A,B)**]; charts **(B,C,E,F,H,I)** - lowercase letters express differences among variants with an equal crop (differing in the date of harvest), uppercase letters express differences among variants discriminated according to the crop type; results based on Tukey's HSD post-hoc test calculated at a significance level $p \leq 0.05$. Rad = radish; Saf = safflower, Wmu = white mustard.

(Couëdel et al., 2018b). This discrepancy likely reflects that glucosinolate degradation is mediated by enzymes like myrosinase (Al-Turki and Dick, 2003; Huber et al., 1983) rather than ARS.

Taken together, these enzyme responses indicate that microbial N and S cycling can shift without parallel changes in bulk TN, highlighting the importance of process-level indicators. While safflower decomposes slowly, it still supports steady N turnover through urease activity. Crucifer residues, in contrast, showed maturity-dependent differences in protein- and chitin-degrading pathways, with direct implications for the timing of incorporation to optimize N release. Moreover, the interaction between glucosinolate-rich residues and sulfur enzymes suggests that biochemical specificity of plant metabolites must be considered when predicting S dynamics under green manuring.

Conclusion

The results suggest that although residue quality indicators such as lignin, cellulose, and C:N ratio are commonly used to predict decomposition rates, they do not always capture the variability associated with different crop types and maturity stages. For cruciferous residues, larger degradation has often been reported despite moderately recalcitrant fibre fractions, suggesting that secondary metabolites such as glucosinolates may play a role in enhancing microbial breakdown. In contrast, oilseed crops such as safflower have been described as more resistant due to structural complexity and phenolic cross-linking in their cell walls, which can decouple decomposition rates from bulk lignin content. Our findings reinforce and expand upon these observations, showing that safflower residues decomposed less extensively than cruciferous biomass even at lower ADL contents, highlighting the importance of polymer interactions and cell wall architecture beyond simple lignin abundance. Moreover, by comparing early- and late-harvested biomass, this study provides novel evidence that maturity stage can override crop identity in shaping residue quality and subsequent soil responses, an aspect largely overlooked in previous green manure studies. This highlights the need to integrate biochemical residue traits with stoichiometric and enzymatic assays to better predict residue–soil interactions and their implications for nutrient cycling and soil carbon dynamics.

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author contributions

Conceptualization, JH, MB, and AK; Methodology, MB, AK, JS, and OM; Software, TB and JH; Validation, JK, AK, and MB; Formal analysis, JH, JK, and MB; Investigation, JH, AK, JK, MB, AM, and MN; Resources AK, TB, JS, and OM; Data curation, OM, JS, and TB; Writing – original draft preparation JH, JK, and MB; Writing – review and editing, JK, MB, TB, JH, AK, MN, and AM; Visualization, JH and TB; Supervision, MB, JK, AK, MN, and AM; Project administration, AK, JH, and MB; Funding acquisition, AK, JH, and MB. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Authors AK and JS were employed by Agricultural Research, Ltd. Author JH was employed by Agrovyzkum Rapotin, Ltd.

The remaining author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/sjss.2026.16036/full#supplementary-material

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Glossary

ADF	acid detergent fiber
ADL	acid detergent lignin
ADL_NC	ratio of ADL to plant nitrous compounds
ARS	soil arylsulfatase
C	plant carbon content
Cacq	soil carbon acquisition ratio
CEL	cellulose
CF	crude fiber
CH	content of carbohydrates
Cox_N	ratio of soil oxidizable carbon to total nitrogen
DM	dry matter
GLU	soil β-glucosidase
HCEL	hemicellulose
K	soil potassium
Mg	soil magnesium
NAG	soil N-acetyl-β-D-glucosaminidase
Nacq	soil nitrogen acquisition ratio
NC	plant nitrous compounds
p	p-value of statistical significance
P	total soil phosphorus
PCA	Principal component analysis
pH	soil pH
Phos	soil phosphatase
PNP	p-nitrophenol
r	correlation coefficient
Rad	radish
RB	residual biomass
Saf	safflower
TC	soil total carbon content
TN	soil total nitrogen content
Ure	soil urease
Wmu	white mustard