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RESEARCH ARTICLE

Effects of high- and low-phenolic pea and field bean residues at contrasting doses on soil respiration, mineral N dynamics, microbial biomass, and subsequent barley growth

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Abstract

Background and aims Legume residues are important organic inputs, but their effects depend on residue chemistry and application rate. This study examined how pea (*Pisum sativum*) and field bean (*Vicia faba*) residues with contrasting phytochemical compositions influenced soil functioning and subsequent barley growth, assessing how residue quality and dose shape soil responses.

Methods A sandy loam soil was amended with residues from four legume genotypes with contrasting phytochemical profiles but similar C:N ratios, applied at low (5 t ha^{-1}) and high (20 t ha^{-1}) rates. Unamended and mineral fertilization treatments were included for comparison. Barley was then grown in a microcosm setup for 8 weeks, and soil CO_2 efflux, microbial biomass C, inorganic N dynamics, and plant biomass were assessed.

Results High-dose applications substantially increased cumulative CO_2 emissions but led to reduced microbial biomass, lower nitrate availability, and significantly suppressed barley growth, particularly for high-phenolic genotypes. In contrast, low-dose low-phenolic residues increased microbial biomass, promoted nitrification, and supported barley growth comparable with mineral fertilizers. Phenolic-rich residues maintained higher ammonium levels and showed reduced microbial and plant responses, indicating inhibitory effects of certain phytochemicals on soil processes.

Conclusion Residue impacts on soil processes and crop performance depend strongly on both phytochemical composition and application rate. Low-phenolic residues applied at moderate doses have positively impacted microbial functioning, nitrogen cycling, and barley biomass, whereas high doses, especially of high-phenolic material, were detrimental to microbial activity and plant growth. Integrating residue chemistry into nutrient management could enhance the sustainability of legume-based cropping systems.

Keywords Legumes · Residues · Organic amendments · CO_2 · Nitrogen · Soil processes · Phenolics · Barley growth

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Introduction

Legumes play a central role in sustainable cropping systems, functioning both as highly nutritious food and feed crops, and as key contributors to soil functions and health (Duarte et al. 2024). Their presence in cropping systems is particularly valued for biological nitrogen fixation (BNF) (Maluk et al. 2023), which avoids the use of synthetic (mineral) nitrogen fertiliser for their own growth, and also reduces this requirement for subsequent crops too (Iannetta et al. 2016). They also enhance soil health more broadly by stimulating biological activity, improving soil structure, increasing productivity of subsequent crops, and strengthening agroecosystem resilience under climate and resource pressures (Picone et al. 2025).

Among grain legumes, pea (*Pisum sativum* L.) and field bean (*Vicia faba* L.) hold particular agroecological importance in cropped systems (Svanes et al. 2022). Both crops are widely cultivated due to their high protein content, nutritional value, and adaptability to diversified rotations (Duarte et al. 2024). Field bean is notable for its exceptionally strong BNF capacity, often exceeding that of other legumes, making it a valuable crop for restoring soil nitrogen pools and reducing further synthetic nitrogenous fertilizer demand (Liu et al. 2019; Maluk et al. 2023). Pea, likewise, contributes to improved soil structure and rhizosphere microbial diversity, while offering shorter growing cycles that support adaptive rotation planning (Chaudhari et al. 2020). Additionally, both species include current-commercial and heritage genotypes that differ markedly in their biochemical and morphological traits, which potentially influence soil processes in different ways (Peoples et al. 1995; Uhlarik et al. 2022).

Building on these multifunctional roles, the incorporation of legume-derived biomass into soil represents a further pathway through which legumes influence soil functioning (Kebede 2021). While legume residues typically exhibit low carbon-to-nitrogen (C:N) ratios (Tao et al. 2024), suggesting rapid decomposition and nutrient release, their impact on soil processes and plant performance extends beyond stoichiometry (Maluk et al. 2022; Sun et al. 2020). The phytochemical composition of residues, particularly the presence of lignin, tannins, and other phenolic compounds, can significantly influence microbial community dynamics, nutrient cycling, and soil CO_2 efflux (Cao et al. 2024). These compounds may either inhibit or stimulate microbial activity depending on their concentration and interaction with soil conditions, thereby affecting the fate of carbon (C) and nitrogen (N) in the soil (Castells 2008).

Recent advances in soil science challenge the traditional view that residue recalcitrance alone governs decomposition and C persistence. Instead, models such as the Microbial Efficiency-Matrix Stabilization (MEMS) framework emphasize the role of microbial processing and mineral associations in determining long-term soil organic matter formation (Cotrufo et al. 2013). In this context, legume residues, despite their relatively labile nature, may contribute to stable C pools if efficiently transformed into microbial biomass and necromass (Kumar et al. 2018).

However, the potential of legume residues to enhance soil health and crop growth is not uniform (Duarte et al. 2024). Variability in residue dose and phytochemical traits among genotypes introduces complexity into their effects on soil respiration, microbial biomass, N availability, and ultimately plant performance (Liu et al. 2023; Rodríguez et al. 2022). While efforts can be made to select genotypes with similar C:N ratios and contrasting phenolic profiles, natural limitations in residue chemistry constrain the degree of control achievable in field conditions (Sun et al. 2020). This study investigates how pea and field bean residue quality and quantity, specifically phenolic composition and application dose, affect soil processes and the growth of spring barley. We assessed these effects by integrating measurements of CO_2 emissions, microbial biomass, inorganic N dynamics, and plant biomass, in a common soil, under controlled environment conditions.

Material and methods

Soil collection and residue processing

The studied soil was a sandy loam collected from an arable field at Invergowrie, Scotland (lat 56°27′18.9"N, long 3°04′19.7"W), which had previously been under spring barley cultivation and had no history of legume cultivation in the preceding decade. Soils were collected from the 0–20 cm layer after barley harvest, sieved (<4 mm) with roots removed and stored at 4 ºC prior to establishment of treatments.

The main properties of the soil are displayed in Supplementary Table S1.

Legume residues were collected from a nearby field where seven commercial genotypes of both field bean (*Vicia faba* L.) and pea (*Pisum sativum* L.) had been grown in the previous year; and for which all their residues (and grains) had been collected from replicate (n = 6) plots (10 m^2) for each. These included the residues of varieties tested here, and for pea the cultivars were: Rose (*Rose*; high phenolic; Mantara UK Ltd); and Kameleon (*Kam*; low phenolic; Senova, UK). And the field bean cultivars: Fuego (*Fue*; high phenolic; Dalton's Seeds, UK), and Lynx (*Ly*; low phenolic; Norddeutsche Pflanzenzucht (NPZ), UK). At grain harvest, the residues were collected directly from the rear of the harvester into a large 'bulk 1-tonne heavy duty woven [86 × 86 × 85 cm] polypropylene bag. The residues were then dried to constant weight at 70 °C, and representative sub-samples from all replicate plots pooled, and cut into small fragments (1–2 cm) using a 'garden plant residue shredder' (#Bosch 600853670, Shredder AXT Rapid 2200). Subsequently, a residue subsample of each genotype was ground to pass through a 0.25 mm sieve for chemical analysis.

Chemical characterization of residues

Total-C and -N concentrations of the residue samples (*ca*. 5–10 mg) were determined by combustion analysis at the UC Davis Stable Isotope Facility (California, USA), using an Elementar VARIO EL Cube (Elementar Analysensysteme GmbH, Hanau, Germany) elemental analyser (Yeomans and Bremner 1991).

To assess general phytochemical differences among samples, plant material was extracted using 80% (v/v) aqueous methanol and sonicated (Branson 3510, Connecticut, USA) for 1 h. The total phenolic content (TPC) was quantified using the Folin–Ciocalteu method (Singleton et al. 1999), with absorbance measured at 765 ηm on a Synergy H1 multidetection microplate reader (BioTek Instruments, Vermont, USA) operated with Gen5 software. Gallic acid was used as a standard (Bastola et al. 2017) to generate the calibration curve (0.020–0.150 mg mL^{-1}), and the results were expressed as milligrams of gallic acid equivalents per 100 g dwt (mg GAE 100 g^{-1} DW). Lignin content was determined using the acetyl bromide method after four sequential 24 h extractions with methanol, water, acetone, and hexane. Approximately 10 mg of dry sample was digested in 12.5% (v/v) acetyl bromide in glacial acetic acid for 2 h under vigorous stirring, followed by centrifugation to recover the supernatant. The solution was then homogenized with 700 µL of glacial acetic acid, 150 µL of 0.3 M NaOH, and 50 µL of 0.5 M hydroxylamine hydrochloride. Absorbance was measured at 280 ηm using a NanoPhotometer, and lignin concentration was quantified from a calibration curve constructed with purified lignin standards (Hatfield et al. 1999). Results were expressed as grams per 100 g (g 100 g^{-1}). All assays were performed in triplicate.

Experimental design

The collected fresh soil was amended with legume residues from four different genotypes (two contrasting genotypes of pea, and two of field bean) under two incorporation rates, corresponding to 5 and 20 t ha^{-1} (residue dry weight), hereafter referred to as low (L) and high (H) doses, respectively. A control treatment without residue addition was also included. In parallel, a mineral treatment had soil amended with NH_4NO_3 mineral fertilizer, with application rates adjusted to minimize discrepancies and match the N input of the two corresponding residue treatments (35 kg N ha^{-1} and 140 kg N ha^{-1}, L and H dose respectively), in that way establishing low and high mineral fertilization levels.

Unamended and amended soils were packed at a bulk density of 1.2 gcm^3 into microcosms and adjusted to 60% water-filled pore space (WFPS) and maintained by regular (three times each week) additions of deionised water throughout the experiment. The microcosm design was adapted from (Paterson et al. 2008) and consisted of PVC cylinders (17.5 cm height, 10.5 cm diameter) with a nylon mesh base (1 mm thick). Each cylinder contained a removable insert (24.5 cm × 7.0 cm) with side openings to permit root penetration. The upper centimetres of the insert functioned as a respiration collar, creating the headspace and extending above the top of the microcosm.

Microcosms were planted with 4 seeds of spring barley in the outer ring of soil, and later adjusted to three per microcosm (i.e., outside of the respiration collar), representing a typical subsequent crop following legumes. Four replicates were conducted for each

treatment, and microcosms were arranged in a randomized block design within a controlled temperature greenhouse maintained at 20 °C with a 16-h photoperiod. Soil CO_2 efflux was monitored throughout the 56-day incubation.

Soil respiration measurements

Prior to sampling, microcosm headspaces were sealed with gas-tight plastic caps and flushed with N_2 via Luer valve fittings integrated into the lids to minimize background CO_2. Gas flow was regulated to prevent overpressure, and flushing continued until CO_2 concentrations reached < 10 ppm. Valves were then closed, and the microcosms were left sealed for a 2-h accumulation period.

Following incubation, CO_2 concentrations were determined as detailed by (Giles et al. 2023), by withdrawing 10 mL gas samples with a syringe and immediately injecting them into an environmental gas monitor (PP Systems, Amesbury, USA). Syringes and needles were replaced between microcosms to avoid cross-contamination. Measurements were taken on days 1, 3, 7, 9, 14, 21, 30, 42 and 56.

Plant and soil sampling

After 8 weeks of incubation, microcosm plants were destructively harvested. Shoots were cut at the soil surface, oven-dried at 70 °C until constant mass and weighed to determine aboveground biomass. Roots were separated from the soil by sieving and manual removal, thoroughly washed, dried under the same conditions, and weighed to determine their belowground biomass.

To obtain composite soil samples, the entire soil volume from each microcosm was removed and homogenized in a clean plastic bag, following the approach described by (Giles et al. 2023). Subsamples were collected for analyses of soil microbial biomass C and inorganic N. Microbial biomass C was quantified via chloroform fumigation extraction following (Vance et al. 1987). Fumigated and non-fumigated fresh soils (equivalent to 12.5 g dwt) were extracted with 0.5 M K_2SO_4 (50 mL). Organic C concentrations in the extracts were determined using a TOC Analyzer (Model 700, College Station, TX), and microbial biomass C was calculated as the difference between fumigated and non-fumigated extracts, applying a kEC conversion factor of 0.45 (Joergensen 1996). The filtered 0.5 M K_2SO_4 extracts were analysed for inorganic N, namely ammonium (NH_4^+–N) and nitrate (NO_2^-–N), using a Konelab Aqua 20 discrete analyser (Thermo Scientific). Ammonium was quantified using a salicylate–dichloroisocyanurate method (Kempers and Zweers 1986), in which ammonia forms an indophenol blue complex measured spectrophotometrically at 660 nm. Nitrate was quantified using a colorimetric method based on the sulphanilamide–NEDD (Griess) reaction (Gauthama et al. 2020). In this procedure, the sample is acidified and reacts with sulphanilamide to form a diazo compound. This compound couples with N-(1-Naphthyl)ethylenediamine (NEDD) to form a reddish-purple azo dye and the resulting colour change, which is proportional to the concentration of nitrite ions, is measured spectrophotometrically at 550 nm. Calibration curves were generated using certified NH_4^+ and NO_3^- standards, and concentrations were converted to dry-soil values using moisture-content data.

Statistical analysis

Differences in plant biomass, soil microbial activity and N content between unamended and soils amended with either different legume residues or mineral fertilizers were assessed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Statistical significance was determined at $p < 0.05$. All analyses were performed using GraphPad Prism version 9.0 (GraphPad Software, Inc., California, USA). All ANOVA model residuals were checked for normality and heteroscedasticity; data were log transformed when needed. CO_2 fluxes cumulative emissions were determined by using the trapezoid rule and calculating the area under rate time curves over the course of the experiment.

Results

Residue chemistry and phytochemical diversity among legume genotypes

Legume residues revealed consistent levels of total C and N among genotypes within the same species, with no statistically significant differences in C:N ratios, which ranged from 47.3 to 74.7 (Table 1). However, pea genotypes *Rose* and *Kam* exhibited significantly

lower N content compared with their field bean counterparts—*Fue* and *Ly*. These differences were similarly reflected in the lignin-to-nitrogen (lignin:N) ratio.

Significant variation was detected in the phytochemical composition across genotypes from both legume species. Total phenolic content (TPC) was significantly higher in *Rose* (527.7 mg GAE 100 g^{-1} DW) and *Fue*, compared with other genotypes; particularly *Ly* and *Kam*, which showed lower values (148.4 and 215.7 mg GAE 100 g^{-1} DW, respectively). Lignin content, a complex phenolic compound, also varied across genotypes, with *Rose* again exhibiting the highest concentration and *Ly* the lowest. *Rose* and *Fue* were thus confirmed as high-phenolic genotypes, while *Ly* and *Kam* were characterized as low-phenolic.

Soil CO_2 emission dynamics

Soil CO_2 emissions varied significantly among amendment doses during the 56-day incubation period, although no major differences were observed between high- and low-phenolic genotypes receiving the same residue doses (Fig. 1; Supplementary Table S2). The highest cumulative emissions (Supplementary Table S2) were observed for all high-dose applications of legume residues. When cumulative CO_2-C release was corrected for the unamended control and expressed relative to the amount of residue C added, low-dose treatments showed a higher estimated proportion of added residue C mineralised than high-dose treatments (Supplementary Table S3). Across treatments, estimated added residue C mineralisation ranged from 9.1 to 12.1% in low-dose residues and from 6 to 6.9% in high-dose residues. Emission rates generally peaked during the first week and declined progressively thereafter, particularly from the second week onward (Fig. 1). For both high- and low-dose treatments, respiration rates remained relatively stable during the later stages of the experiment. Mineral fertilizer treatments (*Min H* and *Min L*) and the unamended control did not exhibit statistically significant differences and showed the lowest cumulative emissions.

Microbial biomass carbon and nitrogen dynamics in soil

By week 8, soil microbial biomass carbon (SMB-C) varied significantly across treatments involving different genotypes, species, application rates, and mineral fertilizer inputs (Fig. 2). High-dose applications of *Rose* (high-phenolic pea) and *Fue* (high-phenolic field bean) residues resulted in the lowest SMB-C values, nearing 200 mg C kg^{-1} soil, although not statistically different from the unamended control ($p < 0.05$). Low-dose treatments of these same residues (*Rose L* and *Fue L*), on the other hand, showed elevated SMB-C, although the values were lower than those of their low-phenolic counterparts, *Kam L* and *Ly L*, which had the highest SMB-C observed. Soil microbial biomass C remained similar between mineral fertilizer and legume residue treatments overall, with differences depending on both residue quality and the applied amount.

Soil inorganic N, primarily consisting of ammonium (NH_4^+-N) and nitrate (NO_3^--N), was significantly affected by residue dose and residue-phenolic level differences. Genotypes with low phenolic content, such as *Kam* and *Ly*, generally led to higher nitrate concentrations, particularly at low residue doses (Table 2). For example, *Kam L* produced the highest NO_3^--N level for the legume amendments

Table 1 Chemical composition of pea (cvs. Rose and Kam) and field bean (cvs. Fue and Ly) residues, including total C, total N, C:N ratio, total phenolic content (TPC), lignin content, and lignin/N ratio (mean ± SD, $n = 3$)

Genotype	Total C (g kg^{-1})	Total N (g kg^{-1})	C:N ratio	TPC (mg GAE 100 g DW^{-1})	Lignin (g 100 g^{-1})	Lignin/N ratio
Rose	378.9 ± 42.7^a	5.30 ± 0.20^a	71.5 ± 10.3^a	527.7 ± 5.7^a	15.06 ± 0.65^a	28.4 ± 1.6^a
Kam	409.6 ± 15.1^a	5.48 ± 0.32^a	74.7 ± 5.2^a	215.7 ± 2.0^b	12.97 ± 0.54^b	23.7 ± 1.7^b
Fue	423.2 ± 19.4^a	8.95 ± 0.76^a	47.3 ± 4.6^a	246.0 ± 12.2^a	12.58 ± 0.55^a	14.1 ± 1.3^a
Ly	406.3 ± 22.3^a	8.24 ± 0.24^a	49.3 ± 3.1^a	148.4 ± 2.1^b	10.95 ± 0.48^b	13.3 ± 0.7^a

Different letters within a column indicate statistically significant differences among the two different genotypes from the same species ($p < 0.05$)

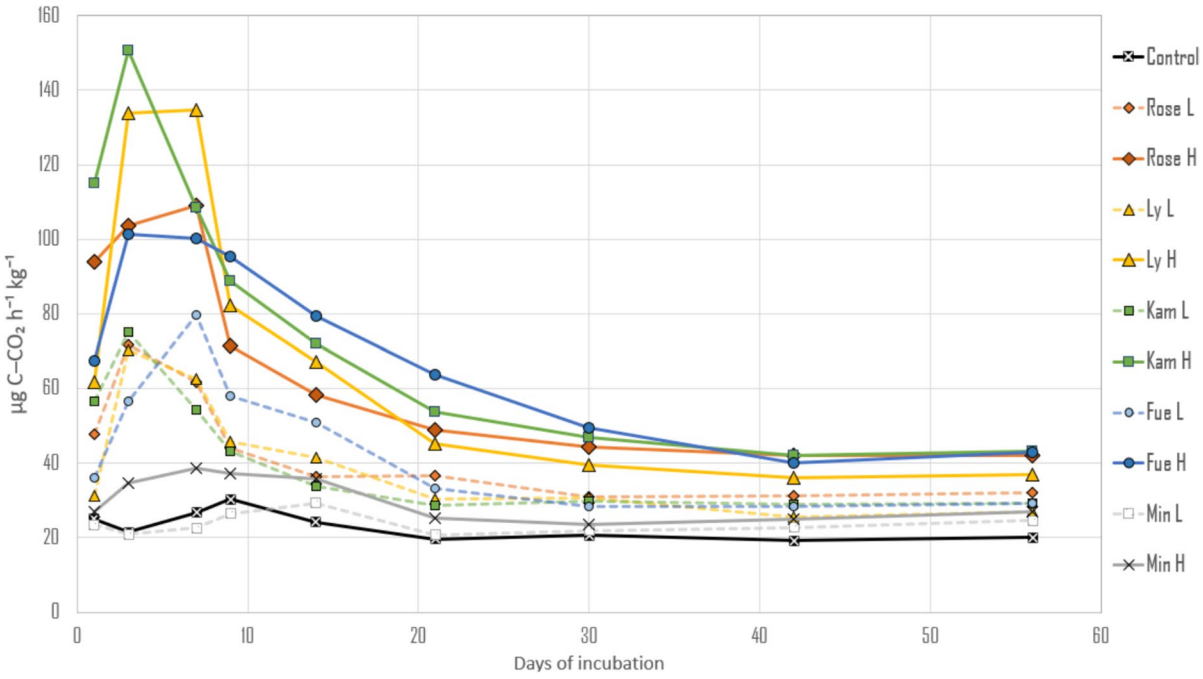

Fig. 1 Temporal trends in soil CO_2–C emission rates (µg C–CO_2 h^{-1} kg^{-1}) over 56 d incubation with pea (cvs Rose and Kam) and field bean (cvs Fue and Ly) residues at low (L) or high (H) doses. Measurements were taken on days 1, 3, 7, 9, 14, 21, 30, 42 and 56. Curves represent mean values for a different legume variety or synthetic (mineral) fertilizer applied at two doses to match that of N residue inputs (Min H, high; Min L, low), along with the unamended control soil

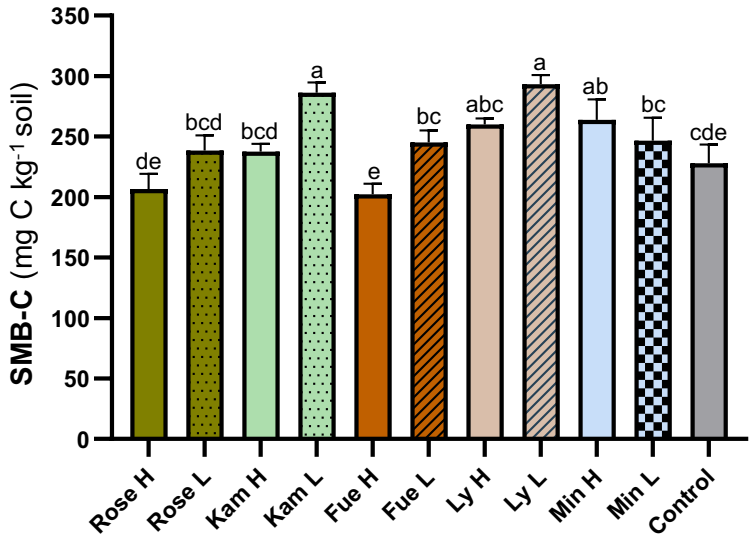

Fig. 2 Soil microbial biomass carbon (SMB-C) across different amendments of pea (cvs. Rose and Kam) and field bean (cvs. Fue and Ly) residues at low (L) or high (H) doses or mineral fertilizer applied at two doses to match for the N residue input (Min H, high; Min L, low), along with the unamended control soil. Values are presented as mean ± standard errors. Different letters indicate significant differences among treatments ($p < 0.05$)

(5.0 ± 1.1 mg/kg), while *Fue H*, a high phenolic genotype, resulted in the lowest (2.0 ± 0.5 mg/kg), highlighting the inhibitory effect of phenolics on nitrification. High residue doses tended to increase ammonium (NH_4^+-N) accumulation, especially in high-phenolic treatments such as *Rose H* and *Fue H*.

Table 2 Soil nitrate (NO_3^--N), ammonium (NH_4^+-N) and total mineral N concentrations (mg/kg), alongside ammonium:nitrate ratio, after 56 days incubation with pea (cvs. Rose and Kam) and field bean (cvs. Fue and Ly) residues at low (L) or high (H) doses or mineral fertilizer applied at two doses to match for the N residue input (Min H, high; Min L, low), along with the unamended control soil

	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	NH_4^+:NO_3^-	Total mineral N (mg kg^{-1})
Control	3.6 ± 0.8^{cd}	1.6 ± 0.5^{bc}	0.44 ± 0.17^{bc}	5.2 ± 0.9^{c}
Rose L	4.0 ± 0.9^{cd}	1.3 ± 0.4^{c}	0.33 ± 0.12^{c}	5.3 ± 1.0^{c}
Rose H	2.5 ± 0.6^{de}	2.3 ± 0.5^{b}	0.92 ± 0.30^{ab}	4.8 ± 0.8^{c}
Kam L	5.0 ± 1.1^{bc}	0.8 ± 0.2^{c}	0.16 ± 0.05^{d}	5.8 ± 1.1^{c}
Kam H	3.5 ± 0.9^{cd}	1.8 ± 0.5^{b}	0.51 ± 0.19^{b}	5.3 ± 1.0^{c}
Fue L	3.5 ± 0.6^{cd}	1.5 ± 0.4^{bc}	0.43 ± 0.14	5.0 ± 0.7^{c}
Fue H	2.0 ± 0.5^{e}	2.5 ± 0.7^{b}	1.25 ± 0.47^{a}	4.5 ± 0.9^{c}
Ly L	4.5 ± 1.0^{c}	1.0 ± 0.3^{c}	0.22 ± 0.08^{d}	5.5 ± 1.0^{c}
Ly H	3.0 ± 0.7^{de}	2.0 ± 0.6^{b}	0.67 ± 0.25^{b}	5.0 ± 0.9^{c}
Min L	13.2 ± 2.5^{a}	4.6 ± 1.1^{a}	0.35 ± 0.11^{c}	17.8 ± 2.7^{b}
Min H	20.4 ± 5.1^{a}	7.1 ± 1.8^{a}	0.35 ± 0.12^{c}	27.5 ± 5.4^{a}

Values are presented as mean ± standard errors. Different letters within each column indicate statistically significant differences between treatments ($P < 0.05$)

This pattern was further reflected in the NH_4^+:NO_3^- ratio, which was highest in Fue H (1.25 ± 0.47), followed by Rose H (0.92 ± 0.30) and Ly H (0.67 ± 0.25), whereas lower ratios were generally observed in low-dose treatments, particularly Kam L and Ly L. Mineral fertilizer treatments consistently showed elevated NO_3^--N levels, indicating rapid N availability compared with organic residues. Total mineral N differed primarily between mineral fertilizer and organic residue treatments, while differences among organic residue treatments were comparatively small.

Effects on barley biomass

Barley biomass varied significantly across treatments (Table 3). The highest aboveground biomass was recorded in the *Min H* treatment (6.91 ± 0.88 g), followed by *Min L*, *Ly L*, and *Fue L*, indicating strong shoot development under mineral N fertilization and low-phenolic low-dose residue treatments. In contrast, high-dose applications of legume residues overall, resulted in markedly lower aboveground barley biomass, with *Rose H* showing the lowest values for barley growth (1.47 ± 0.39 g).

Belowground barley biomass followed a similar trend, with *Min H* again showing the highest root biomass (1.81 ± 0.57 g), while the high-phenolic high dose residue treatments had the lowest values. The shoot:root ratio varied significantly, indicating a more balanced biomass allocation under the low-dose residue applications.

Table 3 Aboveground (AG) and belowground (BG) biomass, and AG:BG ratio after 56 d incubation with pea (cvs. Rose and Kam) and field bean (cvs. Fue and Ly) residues at low (L) or high (H) doses or mineral fertilizer applied at two doses to match for the N residue input (Min H, high; Min L, low), along with the unamended control soil

	Aboveground (AG) biomass (g)	Belowground (BG) biomass (g)	AG:BG ratio
Control	3.91 ± 0.32^{c}	1.32 ± 0.22^{c}	2.96 ± 0.36^{c}
Rose L	4.48 ± 0.86^{cd}	1.6 ± 0.35^{cd}	2.8 ± 0.54^{bc}
Rose H	1.47 ± 0.39^{a}	0.8 ± 0.19^{a}	1.84 ± 0.22^{b}
Kam L	4.67 ± 0.71^{cd}	1.67 ± 0.45^{cd}	2.8 ± 0.25^{bc}
Kam H	2.19 ± 0.54^{b}	1.02 ± 0.22^{ab}	2.15 ± 0.31^{b}
Fue L	4.79 ± 0.87^{c}	1.67 ± 0.31^{cd}	2.87 ± 0.32^{bc}
Fue H	1.77 ± 0.64^{b}	1.1 ± 0.15^{b}	1.61 ± 0.48^{a}
Ly L	5.38 ± 0.74^{d}	1.69 ± 0.29^{cd}	3.18 ± 0.33^{c}
Ly H	1.92 ± 0.61^{b}	0.77 ± 0.11^{a}	2.49 ± 0.55^{b}
Min L	5.27 ± 0.51^{d}	1.54 ± 0.48^{c}	3.42 ± 0.37^{cd}
Min H	6.91 ± 0.88^{e}	1.81 ± 0.57^{d}	3.82 ± 0.42^{d}

Values are presented as mean ± standard errors. Different letters within each column indicate statistically significant differences between treatments ($P < 0.05$)

Discussion

Genotypic variation in nutrient and phytochemical content

In this study, we aimed to maintain comparable C:N ratios across genotypes within the same legume species. Overall, pea had a higher C:N ratio compared with field bean, which was expected given field beans greater BNF capacity (Iannetta et al. 2016). Nevertheless, the observed C:N ratios, ranged from 47.3 to 74.7 and showed no statistically significant differences between same-species types. While relatively high for legumes, these values are consistent with our use of mature post-harvest residues, in which nitrogen remobilisation during senescence leads to higher C:N ratios (Havé et al. 2017). Carbon concentrations (~40%) were not corrected for ash content, which may result in a slight underestimation. However, these values remain within the typical range reported for plant residues (Liu et al. 2022). Thus, we explored additional dimensions of residue quality, particularly phytochemical (TPC) composition, which is often overlooked in similar studies (Schmatz et al. 2017; Truong and Marschner 2020).

Variation in TPC was evident among genotypes, with *Rose* and *Fue* exhibiting substantially higher concentrations than *Ly* and *Kam* (Table 1). Lignin content also varied, with *Rose* again showing the highest levels, which translated into elevated lignin:N ratios. This metric is often used as a proxy for residue recalcitrance (Nakhone and Tabatabai 2008), and the differences observed here may reflect varying dynamics in residue mineralisation rates. These contrasts underscore the substantial phytochemical variability that exists among genotypes, even within the same species (Duarte et al. 2024), suggesting potential functional implications, as phenolics can influence gas-eflux emissions (i.e., soil respiration), microbial activity and so interactions with the following-crop cultivated.

Rates of soil CO_2 emissions under different treatments

The CO_2 emission patterns reflect the influence of residue quantity, but not their biochemical composition, on microbial respiration (Fig. 1). High-dose applications of legume residues consistently resulted in elevated cumulative CO_2 emissions (Supplementary Figure S2), suggesting enhanced microbial activity driven by increased substrate availability. This aligns with previous findings, that organic amendments stimulate microbial respiration by providing labile C sources (Lillo et al. 2025). However, when cumulative CO_2-C release was corrected for the unfertilised control and expressed relative to the amount of residue C added, high-dose treatments showed a lower estimated proportion of added residue C mineralised than low-dose treatments (Table S3). This indicates that the greater absolute CO_2 emissions observed under high residue inputs were largely driven by the larger amount of C added, rather than by a proportionally greater decomposition efficiency. Across residues, only a relatively small fraction of the added residue C was estimated to be mineralised during the 56-day period (Table S3), under the assumption of no positive priming effect. This supports the interpretation that residue decomposition was limited, possibly due to the relatively high C:N ratio of the mature post-harvest residues. Under these conditions, microbial demand for mineral N may have increased during residue decomposition, potentially contributing to temporary N immobilisation or reduced N availability.

It is also important to mention that the high residue dose used here is likely agronomically unrealistic (Liu et al. 2023), but the 5 and 20 t ha^{-1} doses were selected to create clear contrasts in substrate availability, allowing us to isolate the effects of residue quantity on soil processes. While the higher dose exceeds typical field return rates, such elevated inputs are frequently used in microcosm studies (Cao et al. 2024; Engell et al. 2021; Singh et al. 2024) to amplify treatment differences and better understand the observed responses. From an agronomic perspective, these findings suggest that incorporation of mature legume residues with higher C:N ratios may need to occur sufficiently before sowing a subsequent crop, to reduce the risk of temporary N limitation during early crop establishment.

Interestingly, no significant differences were detected between high- and low-phenolic genotypes within the same dose category. This suggests that, under the conditions of this experiment, phenolic content did not exert a strong inhibitory or stimulatory effect on microbial respiration, despite its known role in modulating decomposition processes (Vicena

et al. 2022). It is possible that the concentration or bioavailability of these compounds was insufficient to alter gas-efflux dynamics significantly in this short-term experiment (Zhang et al. 2025). This may be explained by the fact that, even in high phenolic genotypes, these phytochemicals represent only a small proportion of the substrates available to microbes. As a result their effects are likely to be process-specific, for example affecting nitrification, rather than overall CO_2 efflux (Issifu et al. 2024). Additionally, the way residues were applied (chopped tissue) may have influenced phenolic release and accessibility. As plant tissue, even if in smaller fragments, can limit diffusion of phenolics compared with finely processed residues (Dias et al. 2024), potentially further reducing their bioavailability during the experimental timeframe.

The temporal pattern of CO_2 emissions, peaking during the first week and declining thereafter (Fig. 1), reflects typical microbial responses to organic inputs (Baloch et al. 2025). The initial surge likely corresponds to the rapid decomposition of easily degradable compounds, followed by a stabilization phase as more recalcitrant materials dominate and microbial activity slows (Tian et al. 2016). Mineral fertilizer treatments and the unamended control exhibited the lowest cumulative emissions, with no statistical differences between them. Moreover, organic residue amendments are also known to generate not only elevated CO_2 but potentially higher emissions of nitrous oxide (N_2O) (Singh et al. 2024), particularly under conditions that favour nitrification and denitrification (Iannetta et al. 2016). While N_2O was not measured here, its potential relevance underscores the need for future assessments of other GHG fluxes.

Microbial biomass and nitrogen dynamics response to residue quality and quantity

The variation in C from soil microbial biomass across treatments underscores the influence of both residue quality and application rate on microbial responses. High-dose applications of high-phenolic genotypes (*Rose H,* and *Fue H*) resulted in the lowest SMB-C values, which were not significantly different to those of the unamended control (Fig. 2). This supports the idea that higher soil respiration does not necessarily equate to higher microbial biomass (Zhang et al. 2024) and suggests that elevated phenolic concentrations may exert an initial inhibitory effect on microbial growth, potentially through antimicrobial properties or stoichiometric imbalance, slowing residue decomposition and nutrient release (Castells 2008). In contrast, low-dose applications of the same high-phenolic residues supported higher SMB-C, though still lower than their low-phenolic counterparts, *Kam* and *Ly*. The highest SMB-C values were effectively observed in these low-phenolic treatments, reinforcing the view that residue composition, particularly phenolic content, plays a critical role in shaping microbial biomass. SMB-C levels were comparable between mineral fertilizer and legume residue treatments overall, highlighting that microbial biomass can be maintained under both organic and inorganic nutrient regimes. This suggests that the C input from residues did not translate into higher microbial biomass, again evidence of their complexity and gradual decomposition. However, these results should be interpreted with some caution. SMB-C was estimated using chloroform fumigation-extraction with a constant conversion factor (kEC = 0.45) (Joergensen 1996). In treatments containing phenolic-rich residues, interactions between phenolic compounds and extracted organic compounds may have affected extraction efficiency or the relationship between extractable C and microbial biomass (Min et al. 2015). Therefore, the use of a constant kEC may have introduced some uncertainty in SMB-C estimates. Future studies combining fumigation-extraction with complementary approaches, such as phospholipid fatty acid analysis, microbial DNA quantification, or sequencing-based microbial abundance estimates, would help validate these patterns (Joergensen et al. 2024; Petersen et al. 1991).

Soil inorganic N dynamics further support the role of residue quality in shaping not only microbial communities but also nutrient availability. Low-phenolic genotypes, namely *Kam* and *Ly*, resulted in significantly higher nitrate concentrations, in the lower application rates (Table 2). This pattern suggests enhanced nitrification under conditions with fewer inhibitory compounds. Conversely, high-phenolic treatments showed increased ammonium accumulation and reduced nitrate levels, consistent with phenolic-mediated suppression of nitrification processes. This was further reflected in the NH_4^+:NO_3^- ratio, which was highest in Fue H, followed by Rose H and Ly H, suggesting a proportional shift towards NH_4^+

retention relative to NO_3^- under some high-dose residue treatments. Total mineral N, however, did not show a comparable increase across residue-amended treatments, indicating that the main difference was related to the relative distribution between NH_4^+ and NO_3^- rather than to greater overall mineral N availability. These findings are consistent with previous studies showing that phenolic compounds can inhibit microbial activity and form stable complexes with organic N, thereby slowing N transformations (Castells 2008; Chen et al. 2025).

The chemical nature of these compounds, along with soil texture and organic matter, further modulates their effects (Nakhone and Tabatabai 2008). Genotypic differences also influence microbial communities and nitrogen-fixing gene abundance, as shown by (Tao et al. 2024), who reported that legume cultivation enhances microbial N cycling depending on residue quality. However, it is important to note that the plant itself acts as a sink for mineral N, meaning that soil nitrate pools reflect both microbial processes and plant uptake (Zayed et al. 2023); thus, nitrate does not necessarily accumulate even when nitrification is occurring. Additionally, we did not quantify any steps of denitrification, which can represent a major loss pathway for N. Because water-filled pore space in the range of ~ 60% can allow denitrification (Davidson 1993), it is hypothetically possible that higher nitrification in the high-phenolic treatments could have been offset by simultaneous NO_3^- losses via denitrification, thereby masking differences in nitrate accumulation. While this scenario is not the most likely interpretation, it remains an important caveat to our conclusions and highlights the need for future work to quantify gaseous N losses. In our study, *Ly L* yielded the highest nitrate levels overall, supporting the broader conclusion that low-phenolic residues tend to facilitate mineralization and nitrification, while recognizing these limitations.

Mineral fertilizer treatments exhibited consistently higher nitrate concentrations, demonstrating the rapid availability of inorganic N compared with organic amendments (Table 2). Nevertheless this study represents a short-term incubation, and it is well established that mineral fertilizers do not contribute to sustained improvements in soil biological functioning, as higher nitrification alone is not inherently beneficial if it increases the risk of N loss rather than supporting efficient N uptake (Iannetta et al. 2016). Our findings highlight the need for longer-term studies to evaluate the effectiveness of legume residues, including those with higher phenolic content or greater recalcitrance, in supporting nutrient availability and plant growth across several cropping cycles (crop sequences, or rotations). Integrating residue chemistry and microbial responses into nutrient management strategies, as proposed by (Gabbrielli et al. 2024), may enhance the sustainability and efficiency of agricultural systems.

Barley growth

Barley biomass responses across residue treatments demonstrated the capacity of legume-derived amendments to support plant growth at levels comparable with mineral N fertilizers. Notably, low-dose applications of low-phenolic residues resulted in aboveground biomass values that were not statistically different to those observed under low-dose mineral N fertilization (Table 3). This indicates that, when residue quality is favourable and application rate is moderate, residues may mineralise sufficiently to partially support early crop N demand without causing excessive microbial N immobilisation.

In contrast, high-dose applications of legume residues, particularly those with high phenolic and lignin content, were associated with significantly reduced biomass. This outcome is consistent with the literature (Castells 2008) and earlier observations of suppressed microbial biomass and lower nitrate availability in similar treatments, thereby limiting nutrient availability to plants (Zhou et al. 2025). It is also important to recognise that barley preferentially uses ammonium (Macduff and Jackson 1991), and because microorganisms immobilize NH_4^+ more readily than NO_3^- (Ma et al. 2021), higher microbial demand and enhanced nitrification in these treatments may further restrict the forms of N most accessible to the crop. At higher application rates, the resulting mismatch between residue decomposition dynamics and plant N requirements likely limited nutrient acquisition, culminating in the lower biomass observed. However, their slower nutrient release and gradual breakdown may have the potential to enhance N availability and, therefore, plant yield in subsequent cropping cycles as reported by (Cao et al. 2024), though this effect was not evaluated in the present study.

The shoot:root ratio data further support this interpretation, showing more balanced biomass allocation

under low-dose residue treatments (Table 3). This may reflect improved root development in response to the gradual release of nutrients and more favourable soil biological conditions (Pandey et al. 2021). Conversely, the lower shoot:root ratios observed in several high-dose residue treatments indicates the greater plant investment in roots comparing to aboveground biomass, under N-limited conditions, a common response when nutrient acquisition becomes more constrained. This supports the interpretation that high residue doses may have promoted stronger N immobilisation and reduced short-term N availability for barley. We also observed the continued trend of impaired growth with reduced root biomass in high-dose treatments, similarly to other mesocosm studies (Reid et al. 2022).

Taken together, these findings highlight the importance of grain legume residue qualities and quantities in shaping soil biological processes and subsequent crop performance (Baloch et al. 2025). While mineral fertilizers provide immediate nutrient availability, legume residues, particularly those which offer less-recalcitrant mineralisation rates (low phenolic types), and applied at appropriate doses, can better support (compared with high TPC types) microbial activity, nitrification processes, and maintains crop growth in the short-term. Nevertheless, the ecological significance of these conclusions requires further investigation, particularly under field conditions where multiple factors interact – including over crop-sequences (time)—to shape the impacts of residues.

Conclusion

This trial was designed to gain a more holistic and crop-sequence based approach with the aim of providing a more nuanced understanding of grain legume residue qualities and phytochemical diversity in relation to their influence on soil functions, and subsequent (non-legume) crop growth. Low-phenolic residues, when applied at appropriate doses, increased microbial (C) biomass, soil respiration, enhanced N availability, and significantly enhanced crop (barley) growth (biomass production); performing comparably with mineral N fertilizers. In contrast, high residue doses showed inhibitory effects on microbial biomass and crop plant growth, underscoring the importance of both residue composition and application rate. Phenolic content appeared to influence N transformation more clearly than overall soil respiration, particularly by altering the balance between ammonium and nitrate. These findings highlight the importance of optimizing residue incorporation timing before sowing, especially for residues with higher C ratios or slower decomposition (higher phenolic), to reduce potential competition for mineral N between decomposer microorganisms and the following crop.

Although this study aimed to isolate the effects of phytochemical composition, natural variability among residues means that other biochemical and stoichiometric traits cannot be fully disentangled (Baloch et al. 2025). Building on these findings, future research could employ approaches such as stable-isotope tracing to more clearly partition respiration sources, quantify gross N-fluxes, complementary microbial biomass methods such as PLFA or DNA-based quantification and integrate physiological or phylogenetic analyses to resolve underlying microbial processes. These developments would enable a more refined evaluation of genotype-specific residue formulations and their long-term, rotational impacts on soil function, crop productivity, and agroecosystem resilience.

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Data availability The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Declarations

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

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