JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

CROP RESIDUE TYPE AND WETTING AGENTS SHAPE SOIL NUTRIENT TRANSFORMATION AND MICROBIAL FUNCTION DURING DECOMPOSITION

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https://doi.org/10.55251/jmbfs.13473

ARTICLE INFO

Received 30. 10. 2025
Revised 16. 4. 2026
Accepted 24. 7. 2026
Published 1. 8. 2026

Regular article

OPEN ACCESS

ABSTRACT

A 19-week pot study with unplanted arable topsoil evaluated the decomposition of six incorporated cover crop biomasses such as Egyptian clover, pea, phacelia, sunflower, safflower, and white mustard. The biomasses were pre-rinsed with water (control) or with two non-ionic wetting agents including Mero 33528 (rapeseed fatty acid methyl esters + tridecyl alcohol ethoxylate) and Trend® 90 (isodecyl alcohol ethoxylate). Mass loss of biomasses was significant, ranging from ~90% (safflower) to ~60% (sunflower, white mustard). Wetting agents did not systematically enhance the decomposition but redirected the microbial functioning. Mero consistently enhanced enzyme activities, increased total soil N, and lowered microbial C-limitation indices. By contrast, Trend® 90 dampened the enzymatic activity and exerted neutral or negative effects on N cycling and stoichiometric indicators. Both agents reduced bacterial biomass compared to water-rinsed residues, while fungal responses were crop-dependent, consistent with soil C gains observed under Mero. Despite the primary hypothesis, not the wetting agents but the residue quality (C:N ratio) was the primary driver of decomposition efficiency, while wetting agent chemistry modified microbial pathways without materially altering the bulk mass loss.

Keywords: green manure, plant quality, biomass degradation, soil enzymes, microbial biomass

INTRODUCTION

Green manuring of cover crops provides economic benefits (**Schnitkey *et al.*, 2023**). Among others, it improves biomass production (**Antosh *et al.*, 2022**) and delivers ecological services such as the formation of soil organic matter (SOM) and enhanced soil physical, chemical, and biological properties (**Saleem *et al.*, 2020; Arruda *et al.*, 2021; Adetunji *et al.*, 2020; dos Santos Cordeiro *et al.*, 2021; Feng *et al.*, 2021**). Incorporation of green manure into the soil further improves the quality of SOM, and organic carbon content (**Bogužas *et al.*, 2015**), increases organic nitrogen (ON) levels (**Liu *et al.*, 2020**), reduces nutrient leaching (**Couëdel *et al.*, 2018**) and enhances nutrient availability (**Su *et al.*, 2022**).
The efficacy of green manuring depends on the processes following the incorporation into the soil through tillage (**Hu *et al.*, 2023**; **Li *et al.*, 2024**; **Zhang *et al.*, 2023**). Buried plant biomass biodegrades, which is connected with chemical transformation reactions, mainly oxidation (**Li *et al.*, 2008**), nutrient release (**Li *et al.*, 2008; Ranaivoson *et al.*, 2022; Xie *et al.*, 2022**), increase in microbial and enzymatic activity **(Xu *et al.*, 2023; Piotrowska-Dlugosz *et* Wilczewski, 2020; Xu *et al.*, 2023),** and ultimately organic matter mineralization (**Zhang *et al.*, 2023; Li *et al.*, 2018; Xie *et al.*, 2022; Sharma *et* Ghosh, 2000**). Nutrient release is influenced by the composition and stoichiometric ratios of plant residues, particularly by the C:N ratio, which affects whether the nutrient release is accelerated or delayed (**Ranaivoson *et al.*, 2022**). Enhanced enzymatic activity improves nutrient availability and content (**Xu *et al.*, 2023; Solangi *et al.*, 2019; Dong *et al.*, 2021**) and short-term use nutrient efficiency (**Muhammad *et al.*, 2022; Wang *et al.*, 2025**).
The rate of residue biodegradation depends on many factors. Apart from factors such as moisture, temperature, oxygen availability (**Yu *et al.*, 2024**), soil type/texture or soil–residue contact (**Garnier *et al.*, 2008**), an important factor is also the chemical quality of buried crops (**Domagała-Świątkiewicz *et* Siwek, 2022; Khan *et al.*, 2020; Xu *et al.*, 2023**). The buried biomass composition directly influences the soil microbial biomass, community composition, and microbial activity (**Khan *et al.*, 2020; Xu *et al.*, 2023**). Several studies consistently highlight the contrasting effects of crop type: legumes promote gram-negative bacteria and *Glomeromycota* fungi, whereas cereals and grasses (*Poaceae*) stimulate gram-positive bacteria and *Ascomycota* fungi (**Khan *et al.*, 2020; Xu *et al.*, 2023**). Moreover, cereal green manures (e.g., barley) enhanc soil hydrolases involved in C cycling, whereas legumes (e.g., hairy vetch) promote enzymes involved in N and P cycling, reflecting differences in the chemical composition and C:N ratios (**Khan *et al.*, 2020**). Barley green manure decreases oxidative enzyme activities and increases the fungi:bacteria ratio, suggesting enhanced C storage (**Khan *et al.*, 2020**). Similarly, ryegrass green manure increases SOM and reduces catalase activity, accompanied by higher microbial α-diversity in silty soils (**Wang *et al.*, 2024**). In contrast, ref. (**Wang *et al.*, 2024**) also reported a higher pH, available N, P, K, and urease activity under legume green manuring compared to ryegrass. **Ma *et al.* 2021** found that legumes improve soil hydrolysable N, nitrate N, and available P more effectively than non-legumes. Differences also exist among the legume types: *Mucuna* enhanced microbial biomass and Ca content, whereas *Crotalaria* promoted lower microbial abundance but increased soil pH, P, and K (**Nilza *et al.*, 2015**).
Thus, the dependence of plant biomass decomposition in the soil on the type of incorporated crop and its chemical and nutritional composition has been relatively well studied. However, in reality, green manuring biomass is frequently accompanied by various chemicals commonly used in agriculture, which may further alter the plant biomass decomposition and the overall efficacy of green manuring. One particular group of such compounds are wetting agents used as surfactants/spreading agents or emulsifiers (**Kintl *et al.*, 2021**), applied to improve the efficacy of fertilisers and pesticides (**Kovalchuk and Simmons, 2021**). In fact, combining the wetting agents with fertilisers or pesticides enhances the adhesion of spray-applied droplets to plant leaf surfaces (**Zhang *et al.*, 2017; Zhu *et al.*, 2018**).
Wetting agents can alleviate soil water repellency by modifying soil aggregate stability (**Kintl *et al.*, 2021**), and thereby reducing soil erosion (**Krammes *et* Osborn, 1969**). By altering the soil hydrophysical conditions, they influence seed germination (**Osborn *et al.*, 1967**), moisture penetration and level, microbial community composition (**Song *et al.*, 2019**), and nutrient retention (**Chang *et al.*, 2020**). Many wetting agents are organic carbonaceous compounds (e.g.,

derivatives of palmitic or oleic acid, or polyols such as isodecyl alcohol) that can be readily utilized by soil microbiota.

Nevertheless, while the effects of wetting agents on the plant growth are documented (**Reza Nemati *et* Jeong, 2017**), their role in nutrient transformation processes following green manure incorporation remains scarcely studied (**Das *et al.*, 2019**), with no direct investigations available. **Zheng *et al.*, 2016**, concluded that wetting and consequently also soil repellency are important parameters affecting biodegradation and SOM dynamics. As the wetting agents decrease the water repellency of plant biomass, they are expected to enhance the biomass decomposition rate and the nutrient dynamics, which is the main hypothesis of this work. In particular, this research is based on the use of wetting agents (chemically rapeseed fatty-acid methyl esters + tridecyl alcohol ethoxylate and isodecyl alcohol ethoxylate), which function as non-ionic surfactants, as this type of compounds decreases the surface tension or interfacial tension between two liquids and exerts positive effects on soil properties (**Kostka *et al.*, 2007; Silva *et al.*, 2024**). Effects of the tested wetting agents on the soil are expected to be purely physicochemical, as they do not introduce enzymes or microorganisms that could directly stimulate plant residue decomposition. Based on that, the objective of this study was to evaluate the short-term combined effects of wetting agents and green manuring with different crops (incorporated as above-ground biomass) on the soil chemical and biological properties, and to relate these effects to the qualitative traits and nutrient composition of plant biomass. This objective was addressed in a 19-week box experiment conducted in unplanted arable topsoil.

MATERIAL AND METHODS

Soil preparation, specification of crops and wetting agents

The experiment was conducted at Agricultural Research, Ltd. (Troubsko, Czech Republic; 49°17′49.822″ N, 16°49′13.044″ E). The source arable soil was managed under a conventional system with the crop rotation comprising cereals (winter wheat, spring barley), rapeseed, and pea. Climatically, the site is moderately warm and moderately dry, with a mean annual air temperature of 9.0 °C and mean annual precipitation of 526 mm. Geologically, the area is underlain by loess and loess loam of the Bohemian Massif, and the soil was a modal brown earth, classified as a silty clay loam (USDA Textural Triangle) Haplic Luvisol (WRB soil classification), sampled (0–15 cm) near the town Troubsko, Czech Republic (49°10'28"N 16°29'32"E). The chemical properties of the soil 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. The collected soil was homogenized, passed through a 2mm sieve (Retsch GmbH, Germany).

Aboveground plant biomass used for the pot trial was harvested after 77 days (11 weeks) of growth from fields located near Nová Ves, Brno, Czech Republic (49°06′25.7″ N, 16°18′15.3″ E), under conditions (growth phase according to BBCH scale) specified in Table 1. The biomass was oven-dried at 60 °C to constant mass in a laboratory drying oven. The tested crops are listed in Table 2, their dried biomass was initially analysed for qualitative properties (displayed in the Results section).

Table 1 Cover crops used in the pot trial

#	acronym	Cover crop species	Sowing date	Harvest date	BBCH value
1	Ec	Egyptian clover (*Trifolium alexandrinum* L.)	16 August 2021	1 November 2021	33
2	P	pea *(Pisum sativum* L.)	16 August 2021	1 November 2021	65
3	Ph	phacelia (*Phacelia tanacetifolia* Benth.)	16 August 2021	1 November 2021	61
4	S	sunflower (*Helianthus annuus* L.)	16 August 2021	1 November 2021	63
5	Sa	safflower (*Carthamus tinctorius* L.)	16 August 2021	1 November 2021	36
6	Wm	white mustard (*Sinapis alba* L.)	16 August 2021	1 November 2021	72

Two wetting agents (WAs) were evaluated in the pot trial: Mero 33528 (W1; Novance S.A., France) and Trend® 90 (W2; DuPont de Nemours S.A.S., France). Mero 33528 consisted of rapeseed fatty-acid methyl esters (81.4% w/w) and tridecyl alcohol ethoxylate (~7 EO; <20% w/w), while Trend® 90 contained isodecyl alcohol ethoxylate (90% w/w). Both WAs were used as spraying mixtures, prepared by diluting 0.33 mL (of WA) in 100 ml of distilled water, according to the manufacturer's recommendations. For each 15 g portion of dry plant biomass (and for 50 kg of soil, respectively), a working solution was prepared by mixing 50 mL of distilled water with 3.3 mL of the spraying mixture of wetting agent (either W1 or W2). Prior to incorporation into the soil, the plant biomass was treated as follows: nW-rinsed with 50 mL distilled water (no wetting agent); W1-rinsed with 53.3 mL of the Mero 33528 working solution (50 mL water + 3.3 mL of spraying mixture); W2-rinsed with 53.3 mL of the Trend® 90 working solution. This treatment and dosage was adopted in agreement with the previous study of **Kintl *et al.*, 2022**. Therefore, the final dose of each respective concentrated WA was approx. 0.01 $mL \cdot kg^{-1}$ of soil, which equals 0.436 $L \cdot ha^{-1}$.

Experiment design

The pot trial was conducted in rectangular boxes (flat crates; footprint 57 × 37 cm), filled with sieved soil (10 kg per a box), to a depth of 4 cm. Two semipermeable polyamide mesh bags per box (Uhelon 130 T; pore diameter d = 42 nm; SILK & PROGRESS, Czech Republic), each sized 45 × 20 cm and containing 15 g (dry mass per bag i.e. 1.5 $g \cdot kg^{-1}$ of soil) of pretreated plant biomass of a given crop (Table 2), were placed on the soil surface and then covered with a 2 cm layer of soil (see Figure A1). Each treatment variant (Table 2) was prepared in triplicate (n = 3).

Table 2 Experimental variants (their acronyms) according to buried crops and WA treatment

Buried cover crop	no WA	W1 (Mero)	W2 (Trend)
-	Cont	-	-
Egyptian clover (*Trifolium alexandrinum* L.)	nW+Ec	W1+Ec	W2+Ec
pea *(Pisum sativum* L.)	nW+P	W1+P	W2+P
phacelia (*Phacelia tanacetifolia* Benth.)	nW+Ph	W1+Ph	W2+Ph
sunflower (*Helianthus annuus* L.)	nW+S	W1+S	W2+S
safflower (*Carthamus tinctorius* L.)	nW+Sa	W1+Sa	W2+Sa
white mustard (*Sinapis alba* L.)	nW+Wm	W1+Wm	W2+Wm

The experiment was conducted from 25 October 2022 to 6 March 2023 (133 days; ~19 weeks) under following conditions: temperature night/day 9 °C/16 °C, watering one day a week with 1.5 L of distilled water, simulating natural field conditions, alternation of drought and moisture.

At the end of the trial, the overlying soil was gently raked off to expose the bags, which were carefully retrieved. Soil was then sampled from the thin layer (0–2 mm) directly beneath the bottom of each bag by scrabbing the superficial thin layer of soil after the removal of each mesh bag. Therefore, the soil in immediate contact with the plant biomass through the semipermeable mesh was analysed. The remaining plant biomass was quantitatively transferred from the mesh bags to paper bags, oven-dried at 65 °C to constant mass, and weighed on an analytical balance to determine residual biomass (RB; $g \cdot bag^{-1}$).

Analyses of plant biomass and soil

Fibre fractions were determined as acid-detergent fibre (ADF) and neutral-detergent fibre (NDF). ADF using an acid-detergent solution containing cetyltrimethylammonium bromide (CTAB) (**Kintl *et al.*, 2022**), and NDF using a neutral-detergent solution containing sodium lauryl sulfate, (**ANKOM, 2006a**) on an ANKOM 220 fibre analyser (ANKOM Technology, USA). Acid-detergent lignin (ADL) was determined according to ISO 13906:2008 (**ANKOM, 2006b**). Hemicellulose was calculated as NDF – ADF and cellulose as ADF – ADL (**ISO_13906, 2008**). Crude protein was measured by the Kjeldahl method using a Kjeltec™ 2300 Analyzer (FOSS, Denmark) (**Rinne *et al.*, 1997**); protein content was calculated as total nitrogen × 6.25. Lipids (= fat content) were determined gravimetrically using the water-cooled Soxhlet's extractor with petrolether and diethyl ether (**ISO_6865, 2000**). 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 (**AOAC, 2023**; **Pomeranz *et* Meloan, 1994**). Ash content was determined according to ISO 6865:2000 (**Marrubini *et* al., 2017**

Soil processing. Freshly collected soil was passed through a 2-mm sieve (Retsch sieve 200 × 50; Retsch, Germany) and partitioned for the respective analyses. Fresh or 4 °C-stored subsamples were used to determine dehydrogenase activity (DHA) by the 2,3,5-triphenyltetrazolium chloride (TTC) method (**García-Ayuso, *et* Luque de Castro, 1999)** with results expressed in $\mu g\ TPF \cdot g^{-1} \cdot h^{-1}$. Air-dried subsamples were analysed for total carbon (TC) and total nitrogen (TN) by dry combustion **ISO 10694:1995** and **ISO 13878:1998**, respectively (**Doi *et* Ranamukhaarachi, 2009**), using a Vario Macro Cube (Elementar Analysensysteme GmbH, Langenselbold, Germany). The C:N ratio was calculated from the sample-level means of TC and TN.

Soil enzyme assays. Aliquots of freeze-dried soil were 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) activities following **ISO 20130:2018**. End-point spectrophotometry (Infinite M Nano, Tecan Trading AG, Switzerland) was performed at $\lambda = 405$ nm for p-nitrophenol (PNP)-releasing assays and at $\lambda = 650$ nm for the urease indophenol reaction. The activities were expressed as nmol $NH_3 \cdot g^{-1} \cdot min^{-1}$ (urease) and nmol $PNP \cdot g^{-1} \cdot min^{-1}$ (other enzymes).

Enzymatic stoichiometry metrics. Nutrient acquisition ratios (C and N) were calculated from the measured activities following **ISO_20130, 2018**:

EQ1 : C acquisition ratio = ln (DHA+GLU) / ln (DHA+GLU+NAG+Ure)

EQ2 : N acquisition ratio = ln (NAG+Ure) / ln (NAG+Ure+Phos)

Microbial resource limitation was further assessed by vector analysis (**Cui *et al.*, 2022**) using equations EQ3 and EQ4:

$$EQ3: Vectorlength = \sqrt{\left(\frac{ln(DHA+GLU)}{ln(Phos)}\right)^2 + \left(\frac{ln(DHA+GLU)}{ln(NAG+Ure)}\right)^2}$$

$$EQ4: Vectorangle(rad) = ARCTG2\frac{ln(DHA+GLU)}{ln(Phos)};\frac{ln(DHA+GLU)}{ln(NAG+Ure)}$$

where ARCTG2 denotes the arctangent.

qPCR. Freeze-dried soil (0.5 g) was used for DNA extraction with the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, USA). DNA was quantified with a NanoDrop One (Thermo Scientific, USA). SYBR Green-based real-time qPCR was run on a CFX96 system (Bio-Rad Laboratories, USA) to quantify bacterial 16S rDNA and fungal 18S rDNA. Primer pairs were 1108F (5′-ATGGYTGTCGTCAGCTCGTG-3′) / 1132R (5′-GGGTTGCGCTCGTTGC-3′) for bacteria (**Moorhead *et al.*, 2016**), and FF390 (5′-AICCATTCAATCGGTAIT-3′) / FR1 (5′-CGATAACGAACGAGACCT-3′) for fungi (**Amann *et al.*, 1995**).

Statistic analyses

All data (plant biomass and quality traits, soil chemical and biological parameters) were analysed in R v4.3.1.61 Pearson's correlation were used to characterise relationships among the treatments and the selected soil properties (**R_Core_Team R, 2022**) Pearson's |r| was interpreted as: 0.5–0.7 = moderate, 0.7–0.9 = high, and >0.9 = very high correlation (**Mendiburu, 2023**). Figures were produced with the ggplot2 package (**Hinkle *et al.*, 2003**).

For inferential comparisons, one-way and two-way analyses of variance (ANOVA) with Type I (sequential) sums of squares were performed at $\alpha = 0.05$ (**Wickham, 2016**), using "FactoMineR" (**Zar, 1984**) and "Factoextra" (**Lê *et al.*, 2008**) as appropriate. Post-hoc pairwise differences among factor-level means were assessed with the Tukey's honestly significant difference (HSD) test ($\alpha = 0.05$). Means are presented ± standard error of the mean (SEM), and treatment contrasts were used to compute factor-level means (**Kassambara *et* Mundt, 2020**).

Model assumptions were evaluated at $\alpha = 0.05$. Normality of residuals was tested with the Kolmogorov–Smirnov and Anderson–Darling tests from nortest. Homoscedasticity was assessed with the Bartlett's test and the Fligner–Killeen test. Diagnostic plots included: residuals versus fitted values, normal Q–Q plots of standardised residuals, standardised residuals versus fitted values, Cook's distance, residuals versus leverage, and Cook's distance versus leverage (**Kassambara *et* Mundt, 2020**; **Faraway, 2014**). Distributional shape was further examined using the D'Agostino's K-squared test for skewness and the Anscombe–Glynn test for kurtosis from the moments package (**Pekár, S.; Brabec, 2016**).

RESULTS

Properties of plant biomass

All reported differences were statistically significant, differences described in the results were evaluated on a significance level $p \leq 0.05$ based on the statistical evaluation described in the Method section.

Chemical and nutritional traits of plant biomass varied significantly among the crops (one-way ANOVA; partial eta-squared; Table A1; Figures 1A-H), percentual differences are listed in Table A2. Carbon content (C, % dry matter, DM) was highest in pea and lowest in Egyptian clover (Ec) and phacelia (P) (Figure 1A). Nitrogen content (N, % DM) peaked in Egyptian clover, followed by safflower (Sa) and white mustard (Wm); and was lowest in phacelia and sunflower (S) (Figure 1B). Consequently, the plant $C:N_{PLANT}$) was the highest in phacelia and sunflower and lowest in Egyptian clover (Figure 1C).

Ash content (% DM) was the highest in Egyptian clover and phacelia and the lowest in pea (Figure 1D). Carbohydrates (CH) were most abundant in pea followed by phacelia, sunflower, safflower, Egyptian clover and white mustard (Figure 1E). Lipid content was the highest in Egyptian clover and pea, followed by phacelia, safflower, sunflower, and white mustard (Figure 1F). Neutral detergent fibre (NDF) was the highest in pea and Egyptian clover, intermediate in phacelia and sunflower, and the lowest in safflower and white mustard (Figure 1G). Cellulose content (CEL) followed a similar pattern, with the highest values in pea and sunflower and the lowest in Egyptian clover (Figure 1H).

Additional compositional parameters (Table A3) further distinguished the biomass types. Acid detergent fibre (ADF), acid detergent lignin (ADL), and crude fibre (CF) were the highest in pea, whereas hemicellulose (HCEL) and nitrogenous compounds (NC) were the highest in Egyptian clover, which simultaneously showed the lowest ADF and CF values. Sunflower exhibited the lowest ADL, together with low CF, HCEL and NC contents.

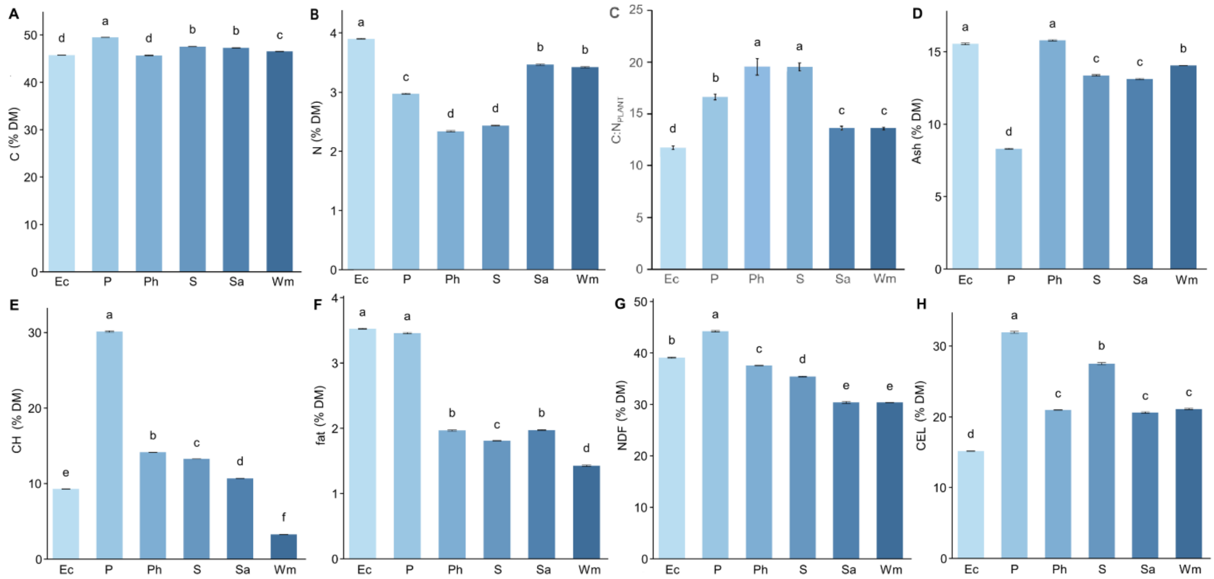

Figure 1 Initial qualitative properties of plant biomass (A-H). Properties: carbon content (C), nitrogen content (N), plant carbon to nitrogen ratio ($C:N_{PLANT}$) Ash, carbohydrate content (CH), fat, neutral detergent fibre (NDF), cellulose content (CEL); displayed are means (n = 3) ± standard error of mean (SEM; error bars); lowercase letters express differences among variants (crop types); results based on Tukey's HSD posthoc test calculated at a significance level $p \leq 0.05$. Ec=Egyptian clover; P= pea; Ph = phacelia; S = sunflower; Sa = safflower; Wm = white mustard.

Mass loss during biodegradation of plant biomass

Residual biomass (RB, g bag^{-1}) after 19 weeks of soil incubation differed significantly ($p \leq 0.05$) among the crops, whereas the effect of wetting agent (WA) was negligible (two-way ANOVA; partial eta-squared; Table A1). Safflower exhibited the greatest mass loss, reflected by the lowest RB across all WA treatments (none, Mero, Trend; Figure 2). By contrast, white mustard showed the highest RB, when averaged across the treatments with wetting agents. Within most crops, RB did not differ among the WA treatments. The only significant WA effect occurred in sunflower, where Mero (W1) reduced RB relative to the no-WA control (nW) (Figure 2).

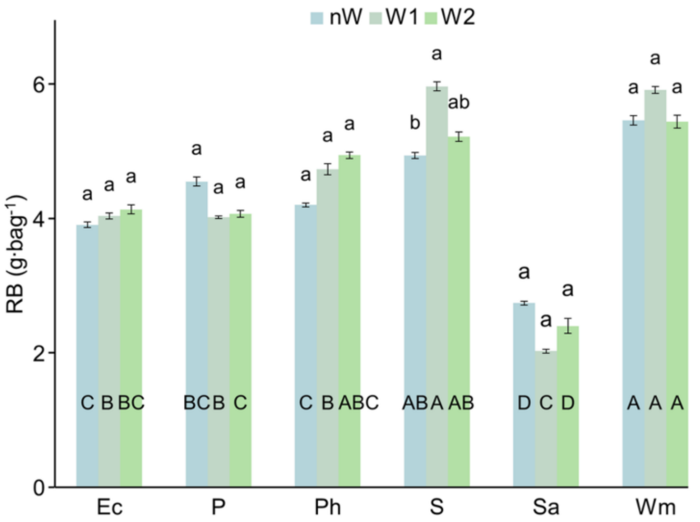

Figure 2 Residual plant biomass (RB). Displayed are means (n = 3) ± standard error of mean (SEM; error bars); lowercase letters express differences in dependence on WA treatment, uppercase letters express differences among variants (crop type) within each group with equally WA-treated; results based on Tukey's HSD posthoc test calculated at a significance level p ≤ 0.05. Ec=Egyptian clover; P= pea; Ph = phacelia; S = sunflower; Sa = safflower; Wm = white mustard.

Table 2 Residual plant biomass (RB) expressed as a percentage of the initially amended amount at the beginning of the trial

Variant	IAB ($g \cdot bag^{-1}$)	RB ($g \cdot bag^{-1}$)	RB_{loss} (% of IAB)
nW+Ec	15.0	3.91	-74%
nW+P	15.0	4.55	-70%
nW+Ph	15.0	4.20	-72%
nW+S	15.0	4.94	-67%
nW+Sa	15.0	2.74	-82%
nW+Wm	15.0	5.46	-64%
W1+Ec	15.0	4.04	-73%
W1+P	15.0	4.02	-73%
W1+Ph	15.0	4.73	-68%
W1+S	15.0	5.97	-60%
W1+Sa	15.0	2.03	-87%
W1+Wm	15.0	5.92	-61%
W2+Ec	15.0	4.14	-72%
W2+P	15.0	4.07	-73%
W2+Ph	15.0	4.94	-67%
W2+S	15.0	5.22	-65%
W2+Sa	15.0	2.40	-84%
W2+Wm	15.0	5.44	-64%

Displayed are means; IAB = initially amended biomass (amount of plants added at the trial start), RB = residual biomass, RB_{loss} = loss of plant biomass due to degradation at the trial end; Ec=Egyptian clover; P= pea; Ph = phacelia; S = sunflower; Sa = safflower; Wm = white mustard.

Soil nutrient content

Soil nutrient status such as total carbon (TC), total nitrogen (TN) (% dry soil), and the C:N ratio, was significantly affected by both the crop variant (crop) and WA (two-way ANOVA with partial eta-squared, Table A1). Percentual differences are listed in Table A2.

Total carbon (TC). In the absence of WA (nW), only the Egyptian clover treatment exceeded the Control (Table A2). Under W1 (Mero), the soil amended with Egyptian clover, pea, and safflower showed a higher TC than the Control. In contrast, no biomass-amended soil exceeded the control under W2 (Trend). Among the crop treatments, Egyptian clover and phacelia had the highest TC under nW. Under W1, the highest TC occurred in pea-amended soil (not different from safflower), whereas under W2 TC did not differ among the crop treatments except for white mustard, which showed the lowest values overall (Figure 3). Comparison across WA treatments revealed a positive effect of Mero: in pea- and safflower-amended soils, TC under W1 > nW and W2 (Figure 3A).

Total nitrogen (TN). All biomass-amended soils had higher TN than the Control. Exception occurred under nW for sunflower and white mustard and under W2 for phacelia, sunflower, safflower, and white mustard (Figure 3B). Under the nW, Egyptian clover produced the highest TN (except for pea). Under W1, the highest TN occurred in pea-amended soil (except for Egyptian clover), whereas under W2 pea again showed the highest values, while sunflower consistently exhibited the lowest TN across all WA treatments (Figure 3B).

C:N ratio. Compared with the Control, the soil C:N ratio decreased in all biomass-amended variants, except for nW+phacelia, nW+sunflower, W1+sunflower, and W2+phacelia/sunflower/safflower (Figure 3C). Under the nW treatment, the pea soil variant had a lower C:N than the soil amended with phacelia, sunflower, and white mustard. Under W1, Egyptian clover showed the lowest C:N (not different from the pea variant), while under W2 the lowest values occurred in pea (similar to Egyptian clover) (Figure 3C). Overall, pea-amended soils consistently showed the lowest C:N across the WA treatments, whereas the phacelia amendment led to the highest values. A within-crop comparison indicated that the W1 reduced the soil C:N relative to nW (and W2 in Egyptian clover), consistent with a Mero-induced shift toward N enrichment (Figure 3C).

The sSoil enzymic activity and indicators of nutrient acquisition and limitation

Activities of all assayed soil enzymes and the derived indicators of nutrient acquisition and limitation varied significantly with crop Variant and WA (p ≤ 0.05; two-way ANOVA; partial eta-squared; Table A1). The percentual differences are listed in Table A2.

Dehydrogenase (DHA). Under the nW and W1 treatments, all biomass-amended variants showed higher DHA than the Control, except nW+pea. In contrast, under W2, most treatments had lower DHA than the Control, except phacelia and sunflower (Table A2; Figure 4A). Within individual crop variants, W1 generally increased DHA relative to nW (Egyptian clover, pea, phacelia, white mustard), whereas W2 reduced DHA in Egyptian clover, safflower, and white mustard soil variantss. Across the crops, sunflower showed the highest DHA under nW and W2 treatments, while under W1,the highest values occurred in pea- and white mustard-amended soils (Figure 4A). Sunflower responses were largely unaffected by W1, whereas pea amendment co-mediated the strongest W1-induced increase in DHA.

β-Glucosidase (GLU). GLU exceeded the Control in the nW treatment with Egyptian clover, pea, and safflower and in W1 with Egyptian clover, pea, phacelia, and safflower. No biomass-amended soils exceeded the Control under W2 (Table A2; Figure 4B). W2 reduced GLU relative to nW in Egyptian clover-, pea-, phacelia-, and safflower-amended soils. Across WA treatments, pea amendment resulted in the highest GLU activity, while sunflower produced the lowest values under nW and W1 (Figure 4B).

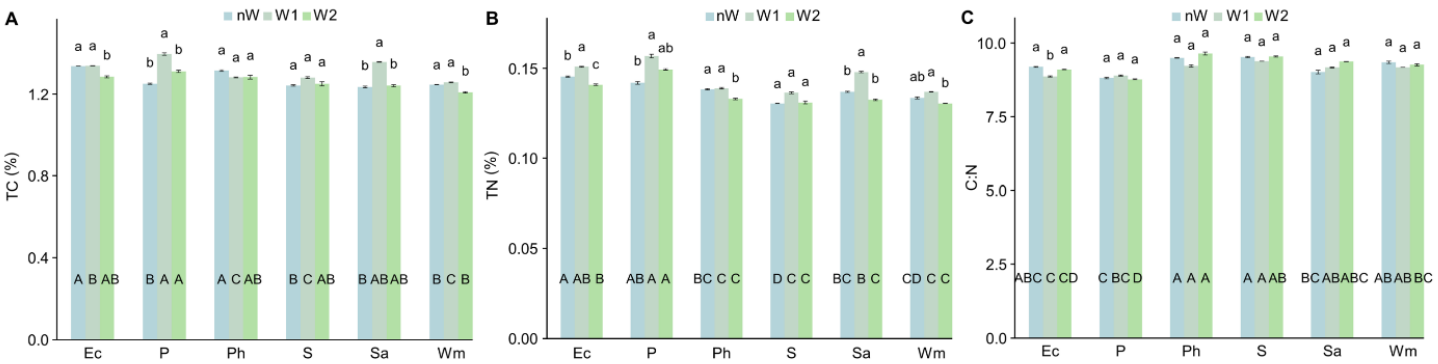

Figure 3 Soil total carbon (TC; A), nitrogen (TN; B), and their ratio (C:N; C) at the end of trial. Displayed are means (n = 3) ± standard error of mean (SEM; error bars); lowercase letters express differences in dependence on WA treatment, uppercase letters express differences among variants (crop type) within each group with equally WA-treated; results based on Tukey's HSD posthoc test calculated at a significance level p ≤ 0.05. Ec=Egyptian clover; P= pea; Ph = phacelia; S = sunflower; Sa = safflower; Wm = white mustard.

N-acetyl-β-D-glucosaminidase (NAG). Biomass incorporation generally increased NAG relative to the Control in all treatments, except nW+sunflower and W2+sunflower (Table A2; Figure 4C). Under nW, Egyptian clover and pea showed the highest NAG activity. Under W1, the highest NAG occurred in the pea-amended soil, while under W2, the highest values were observed for pea and safflower. Sunflower consistently showed the lowest NAG among the biomass-amended soils inall WA treatments. Overall, W1 increased NAG relative to both nW and W2 in most crop-amended soils (Figure 4C).

Urease (Ure). Only nW+phacelia and W2+safflower soil variants exceeded the Control under the nW and W2 treatments, respectively, whereas under the W1 Egyptian clover, pea, phacelia, and sunflower soils showed the Ure activity higher than the Control (Table A2; Figure 4D). Within the crop variants, W1 increased Ure relative to nW in Egyptian clover- and pea-amended soils while W2 reduced Ure in phacelia- and white mustard-amended soils. Under nW, pea-amended soil exhibited the highest Ure activity. Under W1 and W2, safflower and white mustard soil showed lower Ure compared to Egyptian clover and pea. Under W2, Egyptian clover and white mustard soils displayed the lowest activities among W2 treatments (Figure 4D).

Phosphatase (Phos). Phos activity exceeded the Control in pea- and safflower-amended soils under nW and in Egyptian clover-, pea-, safflower-amended soils under W1. In contrast, the Egyptian clover, phacelia, and safflower-amended soils showed a lower Phos than the Control under W2 (Table A2; Figure 4E). Among the crops, pea and safflower exhibited the highest Phos under nW. Under W1, phacelia and sunflower showed lower activities than Egyptian clover, pea, and safflower. Under W2, Egyptian clover, phacelia, and safflower showed a lower Phos than the variant with sunflower. Overall, W2 reduced Phos relative to nW in Egyptian clover-, pea-, phacelia-, safflower-amended soils (Figure 4E).

Arylsulfatase (ARS). Biomass incorporation generally increased ARS under nW and W1 treatments, except for W1+sunflower (Tablee A2; Figure 4F). Under W2, ARS did not differ from the Control. Among the crops, sunflower and white mustard reduced ARS relative to other nW soils, while under W1, the lowest activities occurred in phacelia- and sunflower-amended soils. In all biomass-amended variants, W2 resulted in lower ARS than nW, whereas W1 increased ARS in pea-, safflower-, and white mustard-amended soils (Figure 4F).

Carbon acquisition ratio (Cacq.). Relative to the Control, Cacq. decreased in all biomass-amended soilss under W1 and W2 treatments, except for the white mustard variants (W1 and W2). Under nW, only the phacelia-amended soil showed a lower Cacq than the Control (Table A2; Figure 5A). Within the nW treatments, phacelia and white mustard had a lower Cacq than the pea-amended soil. Under W1, white mustard showed a higher Cacq than Egyptian clover, while under W2, white mustard exceeded the pea-, phacelia-, and safflower-amended soils. Across the crop variants, both W1 and W2 reduced Cacq relative to nW in Egyptian clover-, pea-, safflower-amended soils (Figure 5A).

Nitrogen acquisition ratio (Nacq.). All biomass variants exhibited a higher Nacq. than the control across the WA treatments, except W2+white mustard (Table A2; Figure 5B). Under nW, phacelia showed the highest Nacq (not differing from Egyptian clover). Under W1, the pea-amended soil exhibited the highest Nacq, while under W2 both pea and ranked highest. Compared with nW, W2 decreased Nacq in phacelia- and white mustard-amended soils but increased in the pea- and safflower-amended soils (Figure 5B).

Vector length. Patterns largely mirrored Cacq. Biomass-amended soils under W1 and W2 generally showed a lower Vector length than the Control, except for W1+white mustard and W2+Egyptian clover/white mustard (Table A2; Figure 5C). Under nW, only phacelia showed lower values than the Control. Under W1, white mustard exceeded Egyptian clover and pea, while under W2, white mustard showed the highest values among the crop variants (except Egyptian clover). Across the crops, W2 reduced the vector length relative to nW in Egyptian clover-, pea-, sunflower-, safflower-amended soils (Figure 5C).

Vector angle. Angle showed an inverse pattern to Nacq. Most biomass-amended variants had lower values than the Control across the WA treatments, except W2+white mustard (Table A2; Figure 5D). Under nW, phacelia showed the lowest angle among the crop variants (similar to Egyptian clover). Under W1, white mustard had higher values than Egyptian clover-, pea-, and phacelia-amended soils, while under W2 it exceeded all other variants. Relative to nW, W1 reduced the vector angle in Egyptian clover and pea, whereas W2 reduced it in pea and safflower but increased it in phacelia and white mustard (Figure 5D).

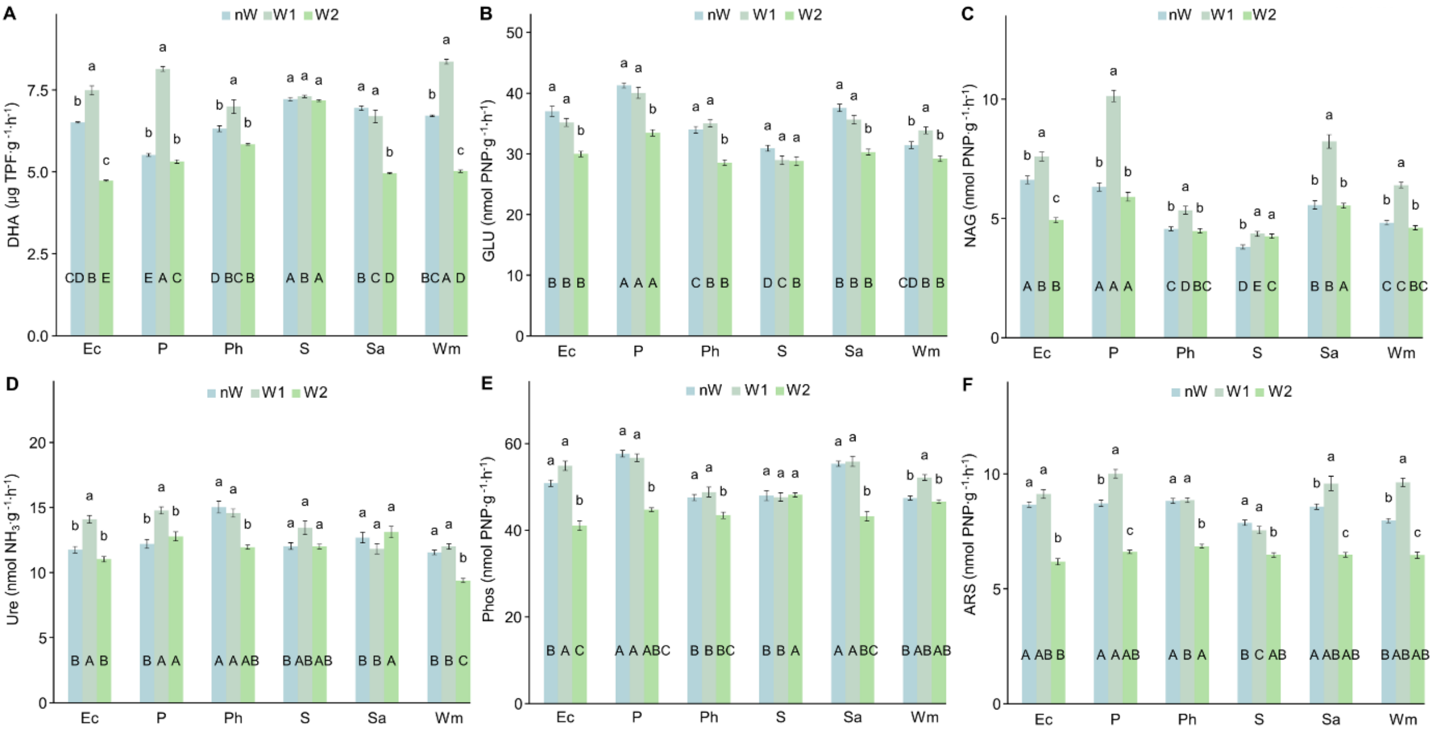

Figure 4 Soil enzyme activities at the end of pot trial. Properties: dehydrogenase (DHA; A), β-glucosidase (GLU; B), N-acetyl-β-D-glucosaminidase (NAG; C), urease (Ure; D), phosphatase (Phos; E), arylsulfatase (ARS; F); displayed are means (n = 3) ± standard error of mean (SEM; error bars); lowercase letters express differences in dependence on WA treatment, uppercase letters express differences among variants (crop type) within each group with equally WA-treated; results based on Tukey's HSD posthoc test calculated at a significance level $p \leq 0.05$. Ec=Egyptian clover; P= pea; Ph = phacelia; S = sunflower; Sa = safflower; Wm = white mustard.

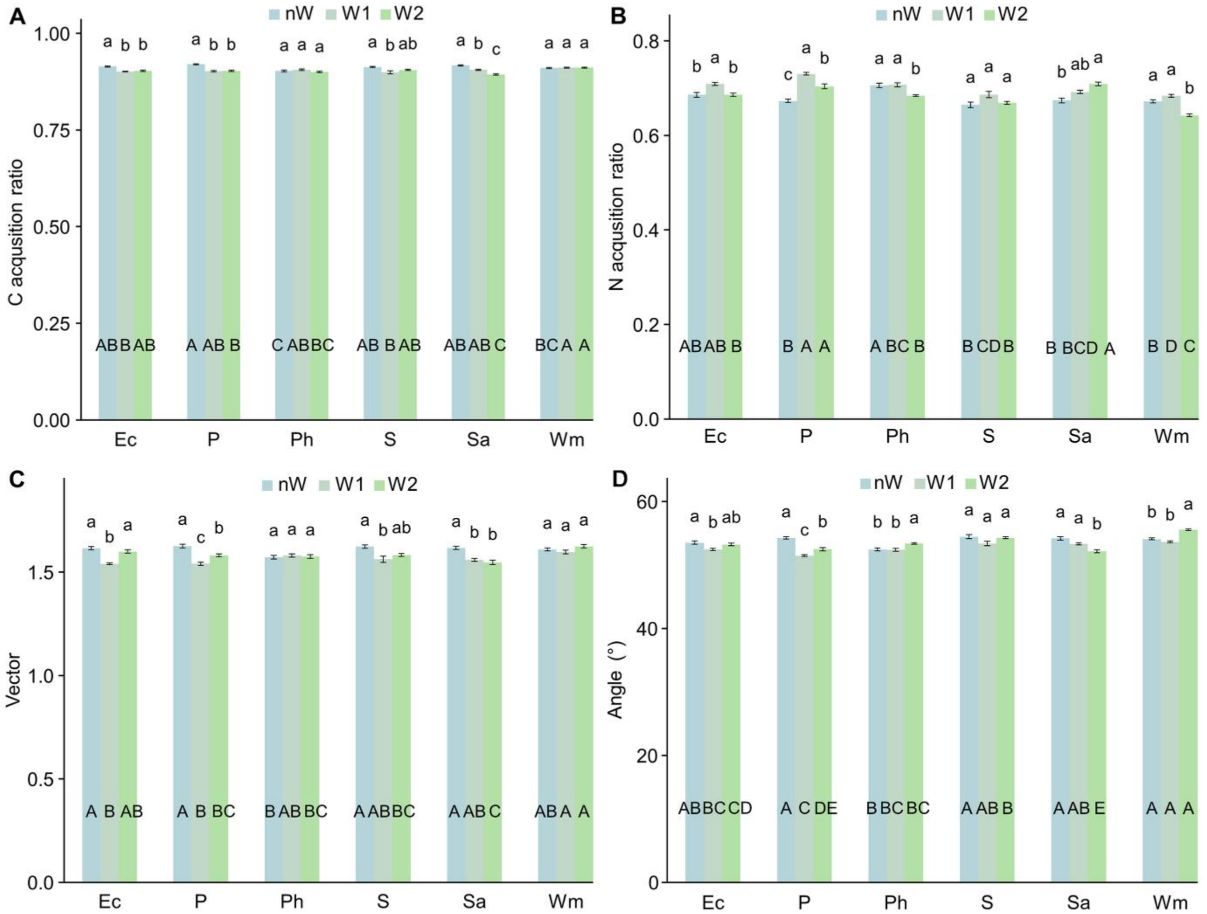

Figure 5 Nutrient acquisition and limitation indicators in the soil at the end of pot trial. Properties: carbon acquisition ratio (Cacq.; A), nitrogen acquisition ratio (Nacq.; B), C limitation index (Vector; C), N/P limitation index (Angle; D); displayed are means (n = 3) ± standard error of mean (SEM; error bars); lowercase letters express differences in dependence on WA treatment, uppercase letters express differences among variants (crop type) within each group with equally WA-treated; results based on Tukey's HSD posthoc test calculated at a significance level p ≤ 0.05. Ec=Egyptian clover; P= pea; Ph = phacelia; S = sunflower; Sa = safflower; Wm = white mustard.

Soil microbial biomass

qPCR-derived microbial biomass, expressed as bacterial (16S rDNA) and fungal (18S rDNA) gene abundance, varied significantly with the crop variant and WA (p ≤ 0.05; two-way ANOVA; partial eta-squared; Table A1). The percentual differences are listed in Table A2.

Bacterial biomass (16S rDNA). Relative to the Control, bacterial biomass increased only under nW in the soil treated with Egyptian clover, phacelia, sunflower, and safflower (Table A2, Figure 6A). Under W2, Egyptian clover-, pea-, and sunflower-amended soils showed lower values than the Control, while white mustard consistently produced the lowest bacterial biomass in all WA treatments. Within the nW treatments, Egyptian clover and phacelia showed the highest bacterial abundance. Under W1, all variants were similar except for a decrease in white mustard. Under W2, phacelia and safflower led to the highest 16S rDNA levels. Across the crop variants, both W1 and W2 decreased 16S rDNA relative to nW, with a more pronounced decrease under W2 for Egyptian clover, phacelia, sunflower, and safflower, with the reduction generally more pronounced for W2 in the Egyptian clover, sunflower, and safflower treatments(Figure 6A).

Fungal biomass (18S rDNA). Fungal biomass showed a greater variability but was generally higher in biomass-amended treatments than in the Control across WA treatments. Exceptions occurred for nW+pea, nW+sunflower, nW+white mustard, W1+white mustard, and under W2 in Egyptian clover, pea, sunflower, and white mustard (Table A2, Figure 6B). White mustard consistently exhibited the lowest 18S rDNA. Under nW, Egyptian clover and phacelia produced the highest fungal abundance, mirroring the bacterial pattern. Under W1, the highest values occurred in the pea- and safflower-amended soils, while under W2, safflower (followed by phacelia) showed the greatest fungal abundance. Comparisons within crop variants indicated that W1 reduced fungal biomass relative to nW in the Egyptian clover-, phacelia-, and sunflower-amended soils (Figure 6B).

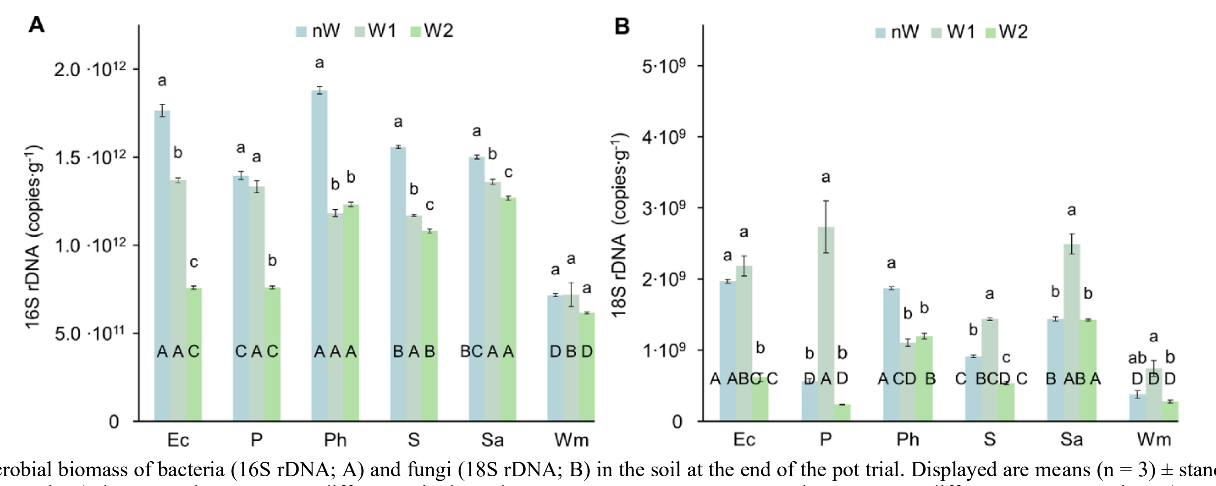

Figure 6 Microbial biomass of bacteria (16S rDNA; A) and fungi (18S rDNA; B) in the soil at the end of the pot trial. Displayed are means (n = 3) ± standard error of mean (SEM; error bars); lowercase letters express differences in dependence on WA treatment, uppercase letters express differences among variants (crop type) within each group with equally WA-treated; results based on Tukey's HSD posthoc test calculated at a significance level p ≤ 0.05. Ec=Egyptian clover; P= pea; Ph = phacelia; S = sunflower; Sa = safflower; Wm = white mustard

Relationships among the soil and plant properties

Figure A2**Figure A2**

DISCUSSION

Impact of plant properties and wetting agents on the loss of biomass through biodegradation

Residual-biomass results (Figure 2) indicated rapid biodegradation, with mass loss ranging from ~ 90% for W1+safflower to ~ 60% for W1+sunflower and W1+white mustard. The results follow the general pattern that legume residues with the lower C:N ratio and lower lignin content decompose faster than residues with higher C:N and rich in ligneous and phenolic compounds (**Komsta *et* Novomestky, 2022**). The relationship between the residue quality control, decomposition rate, and N release is well established. Under field conditions, approximately 50% of legume-derived N may be released within four weeks, and 70-80% within eight weeks; timing varies with the species, maturity at termination, and weather (**Jensen *et al.*, 2005**). When residues are incorporated at appropriate timing, this release window can coincide with the main nitrogen demand of maize during early vegetative stages. Therefore, all biomass types used in this work fulfil the criteria for green manuring with cover crops.
Furthermore, consistent with the classical litter-quality controls (**Dabney *et al.*, 2010**), residues with high nutrient content and lower lignin:N ratios decomposed faster (**Heal *et al.*, 1997**; **Gupta *et* Singh, 1981**). The most decomposed biomasses (safflower, Egyptian clover) contained higher N and nitrogenous compounds (NC) but lower cellulose (CEL) and fibre (ADF, CF) fractions. Conversely, sunflower and white mustard showed lower decomposition rates and contained higher proportions of recalcitrant components such as ash, cellulose and lignin-related fractions. These patterns confirm the importance of lignocellulose composition for biodegradability (**Melilo *et al.*, 1982; Guerriero *et al.*, 2016**). Pearson correlation (Figure A2) further showed that the residual biomass increased with the residue C:N ratio, indicating that stoichiometric constraints rather than specific biochemical fractions primarily controlled decomposition rates under the experimental conditions (Figure A2).
Safflower biomass showed the highest degradability and mass loss. This aligns with the previous reports describing positive effects of safflower green manure on the soil carbon dynamics (**Foston *et* Ragauskas, 2012**), and reflects its relatively high plant carbon content (Figure 1A). Agronomically, safflower is recognized as a drought- and salinity- tolerant catch crop suitable for green manuring (**Kucerik *et al.*, 2024**). By contrast, white mustard exhibited the highest persistence, contributing to relatively low soil TC and TN levels ((Figure 3 A,B) and showing the lowest microbial biomass (bacterial and fungal; Figure 6 A,B). Reduced degradability of white mustard likely results from higher proportions of slowly decomposable polymers such as lignin (**Foston *et* Ragauskas, 2012**), acid detergent lignin (this study) or cellulose (**Foston *et* Ragauskas, 2012**). A similar low microbial activity following the mustard residue decomposition was reported previously (**Foston *et* Ragauskas, 2012)**. Nevertheless, mustard residues can still increase nutrient availability via the weathering of mineral components (**Nayaka *et al.*, 2018**). In contrast to herein presented pot experiment free of planted cover crops, common agromanagement includes the interaction with rhizosphere and variability of conditions which diversely reshape the soil environment and thus, our findings cannot be entirely implicated into the cultivation practice.
Despite their known effects on improving wettability (**McGuire, 2003; Garnier *et al.*, 2008**) and enzyme accessibility (**Qing *et al.*, 2010; Wang *et al.*, 2020**) wetting agents did not significantly accelerate biomass decomposition. Both tested wetting agents are rapidly biodegradable (**Zhou *et al.*, 2019**; **Mösche, 2004**), yet their application produced only minor changes in in decomposition rates. In fact, the decomposition rate slightly decreased under Mero (W1). Instead, decomposition was likely constrained by substrate stoichiometry (as suggested also by Pearson correlation in Figure A2), particularly high C:N ratios, which create microbial nutrient imbalances that wetting agents cannot overcome. Conclusively, the hypothesized primary role of wetting agents in the decomposition efficiency of plant biomass was rejected.
Microbial responses may also explain the limited effects of wetting agents. SSoil microorganisms can utilize fatty-acid methyl esters present in some surfactants, but at low concentrations (**Authority *et al.*, 2022**). Repeated wetting agents applications were shown to alter soil microbial communities and suppress specific taxa (**Song *et al.*, 2019**). In the presented study, the bacterial biomass declined after both wetting-agent treatments (Figure 6A), which may reflect sensitivity of bacterial membranes or biofilms to non-ionic surfactants (**Flykt *et al.*, 2003; Falk *et al.*, 2019**). By contrast, the fungal biomass remained largely unchanged (Figure 6B). This is consistent with reports that many fungi tolerate non-ionic surfactans (**Liu *et al.*, 2023**) or even benefit from them (**Garon *et al.*, 2002; Hodgson *et al.*, 2000**). However, the aforementioned absence of plant biomass interaction with soil rhizosphere and of variable field conditions (which apply in the common agromanagement) in the presented pot experiment (free of growing plants) could also diversely reshape the soil environment, leading to results not fully comparable and applicable to the cultivation practice.

Impact of wetting agents on soil carbon dynamics

Green manuring is often linked to soil TCincreased via sequestration of plant residues (**Li *et al.*, 2008; Ma *et al.*, 2024**), particularly from legumes (**Zhang *et al.*, 2025**). Fungi play a central role in lignocellulose decomposition (**Patra *et al.*, 2023; Cox *et al.*, 2001; Luo *et al.*, 2015**) through the formation of mineral-associated organic matter (MAOM) and aggregation processes (**Miltner *et al.*, 2009**). In this work, wetting agent effects on fungal biomass (Figure 6 and Figure A2) ranged from neutral to positive. In particular, W1 (Mero, FAME + alcohol ethoxylate) strengthened TC accumulation in Egyptian clover, pea, and safflower treatments (Table A2) and coincided with higher fungal biomass (Figure 6B), suggesting enhanced C storage (**Sae-Tun *et al.*, 2022**; **Wang *et al.*, 2024**). This is also supported by two-way ANOVA test (Table A1) in this study and Pearson correlation (Figure A2).
Nevertheless, biomass inputs can also accelerate SOM degradation (**Surucu *et al.*, 2014; Zhang *et al.*, 2019**). In the presented experiment, increased dehydrogenase activity (DHA) under W1 supports enhanced microbial activity, possibly linked to the presence of fatty-acid-based compounds in the surfactant (**Polewczyk *et al.*, 2020; Achuba *et* Okoh, 2014**). In contrast, Trend® 90 (W2) showed neutral or negative effects on DHA in Egyptian clover, safflower, and white mustard. This may reflect lower mineralization rates at higher ethoxylate concentrations (**Gejlsbjerg *et al.*, 2003**) (despite fast biodegradability of ethoxylates (**Talmage, 2020**)) or rapid degradation of W2 before the measurement of the enzymatic activity (after 19 weeks).
Effects on β-glucosidase (GLU) were relatively modest and mainly significant only for white mustard. GLU responses were positively correlated with Cacq. and TN, indicating stronger microbial carbon demand where nutrient availability was balanced. Whereas Mero's effect on GLU was limited, Trend® 90 generally suppressed it, likely due to reduced microbial proliferation. Overall, the co-amendment of biomass with Mero (W1) appeared to support fungal growth, enhance EOM degradation (DHA) and moderately increase N release (**Wang *et al.*, 2025**; **Foston *et* Ragauskas, 2012**). Consequently, slight decreases in C:N ratio and lower C limitation were observed. Therefore, the effect of wetting agents on carbon sequestration after green-manuring was biomass-dependent.

Effects on nitrogen cycling and nutrient limitation

Consistent with the literature (**Wang *et al.*, 2025**; **Lyu *et al.*, 2023)**, incorporation of most biomass types increased soil TN relative to the control (Figure A2). Mero significantly enhanced TN in Egyptian clover, pea, and safflower treatments. In legume treatments, improved N availability likely reduced P limitation and increased N acquisition, consistent with known effect legume green manures (**Lyu *et al.*, 2023; Gatsios *et al.*, 2021**).
Trend® 90 showed weaker or negative effects on nitrogen dynamics and produced inconsistent responses in nitrogen acquisition. Lower fungal biomass in phacelia- and white-mustard–amended soils might have reduced the microbial processing of organic matter and slowed nitrogen cycling(**Gorfer *et al.*, 2014**; **Pánek *et al.*, 2024**).
Enzyme responses further highlighted the importance of fungal biomass. N-acetyl-β-D-glucosaminidase (NAG), involved in chitin turnover, responded strongly to biomass incorporation (except sunflower) and was further stimulated by Mero (vs. nW and W2), suggesting greater fungal necromass cycling (**Xu *et al.*, 2023**; **Cox *et al.*, 2001**). Urease activity also increased in several treatments, particularly in legumes under W1, consistent with previous observations of nitrogen-cycling stimulation by green manures (**Sucuru *et al.*, 2014**; **Balota *et* Chaves, 2010**; **Ye *et al.*, 2014**).
Phosphatase and arylsulfatase activities increased after biomass addition, confirming enhanced phosphorus and sulphur cycling (**Piotrowska-Dlugosz *et* Wilczewski, 2020**; **Khan *et al.*, 2025**; **Ye *et al.*, 2014**; **Tejada *et al.*, 2008**). Mero further stimulated phosphatase, indicating improved P availability (**Katarija *et al.*, 2024**), and arylsulfatase activities in most treatments. In contrast, Trend® 90 suppressed arylsulfatase in all treatments, likely reflecting stronger microbial inhibition at higher ethoxylate levels (**Telmage. 2020**). Overall, nitrogen dynamics were most positively affected in W1 treatments, while W2 showed weaker or negative effects.

CONCLUSION

In six cover crop residues, residue quality, especially C:N, was the primary control on decomposition, with residual mass most strongly correlated to C:N rather than proximate fractions (fat, NDF, hemicellulose). Consequently, the hypothesis was rejected that wetting alone was rate-limiting: pre-rinsing residues with non-ionic wetting agents did not increase mass loss over the 19-week incubation.
By contrast, adjuvant chemistry reconfigured the microbial functioning. The FAME–alcohol-ethoxylate formulation (Mero 33528) consistently enhanced the multi-enzyme activity (dehydrogenase, N-acetyl-glucosaminidase, urease, arylsulfatase), increased soil total N (and, for some residues, total C), and reduced indices of microbial C limitation (enzyme-stoichiometric vectors), pointing to the stimulated nutrient acquisition under the otherwise stoichiometrically constrained conditions. The isodecyl alcohol-ethoxylate (Trend® 90) frequently dampened

enzyme activities and showed neutral to negative effects on N pools and stoichiometric indicators. Both wetting agents reduced the bacterial biomass, while fungal biomass responses were crop-dependent, consistent with a partial shift toward fungal processing pathways under Mero.
Taken together, these results indicate that who processes residue C and N, and how, can be altered without changing the bulk decay rate. For practice, this means: (i) design green-manure mixes to target C:N ≈ 20-30 for synchrony with crop N demand; (ii) do not expect wetting agents to speed residue disappearance; and (iii) recognize that adjuvants can redirect enzyme suites and microbial guilds, with potential implications for N availability and soil-C outcomes.
Our unplanted pot system isolates residue–adjuvant mechanisms but lacks rhizosphere feedbacks and field variability. Therefore, our findings cannot be entirely implicated into the cultivation practice of common agromanagement. Priority next steps include planted field trials, dose-response tests across soils, and community-resolved analyses (e.g., amplicon/PLFA plus extracellular enzyme kinetics) to link enzyme stoichiometry, microbial necromass formation, and mineral-associated C under realistic management.

Acknowledgments: The work was supported by the Ministry of Agriculture of the Czech Republic by project QK21010161, by institutional support MZE-RO1225, MZE-RO1725 and by project VUK-IP-00027073.

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