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Organic amendments influence soil properties, soil microbial diversity, and winter barley traits in a five-year field trial with contaminated soils at a former wood preservation site

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Abstract

Background Soil contamination with metal(loid)s and organic pollutants creates environmental and health concerns, driving the need for sustainable remediation strategies. Organic amendments can mitigate contamination effects, enhancing soil quality, and potentially increasing biomass production; however, their long-term influence remains an open question. In a five-year field experiment at a former wood-preservation site, this study evaluates the effects of five organic amendments—fresh pig manure (PM), biodigested pig manure (PD), compost (C), compost pellets (Pt), and green waste compost (G)—on Cu-contaminated soils. Here, we evaluated their impacts on physico-chemical soil properties, metal bioavailability, microbial community structure, plant growth and soil fertility.

Results All amendments led to an overall soil improvement, including enhanced physico-chemical properties, increased enzyme activities. The amendments promoted the concentration of soil 16S bacterial genes and improved the yield of winter barley cultivated in the plots. The most abundant phyla detected across soil samples were *Actinobacteriota*, *Proteobacteria*, and *Firmicutes*, with *Bacillus*, *Streptomyces*, and *Bradyrhizobium* among the dominant genera. Compost-based amendments at 5% w/w addition rate (C5 and Pt5) showed the most promising results, significantly increasing soil carbon, nitrogen, and phosphorus contents, while reducing bioavailability of Cd, Ni, Pb, and Zn compared with untreated control plots ($p < 0.01$). A decrease in Cu availability was observed but it was not significant. The Pt5 soils exhibited the highest 16S rRNA gene copy number ($p < 0.01$). Both compost and compost pellets amendments enriched microbial communities associated with soil quality and plant yield, leading to significant improvements in soil fertility and barley yield (+ 200% on average).

Conclusion This integrative approach identified organic amendments, notably compost and pelleted compost, that effectively contribute to soil remediation from multiple perspectives: chemical properties (pH, organic content, nutrients), reduction of bioavailable soil Cd and Zn, enzyme activities, microbial abundance and diversity (16S rRNA), and winter barley yield. The study evidenced signature biomarkers characteristic of healthy soils (*Paenibacillus*, *Lysinibacillus*, and *Agromyces*) and polluted soils (*Candidatus Solibacter* and *Mycobacterium*). Our findings support the use of compost (raw and pelleted) as a balanced approach for phyto-managing metal-contaminated soils, reducing 1 M NH_4NO_3-extractable soil Cd and Zn while enhancing microbial activity and soil fertility.

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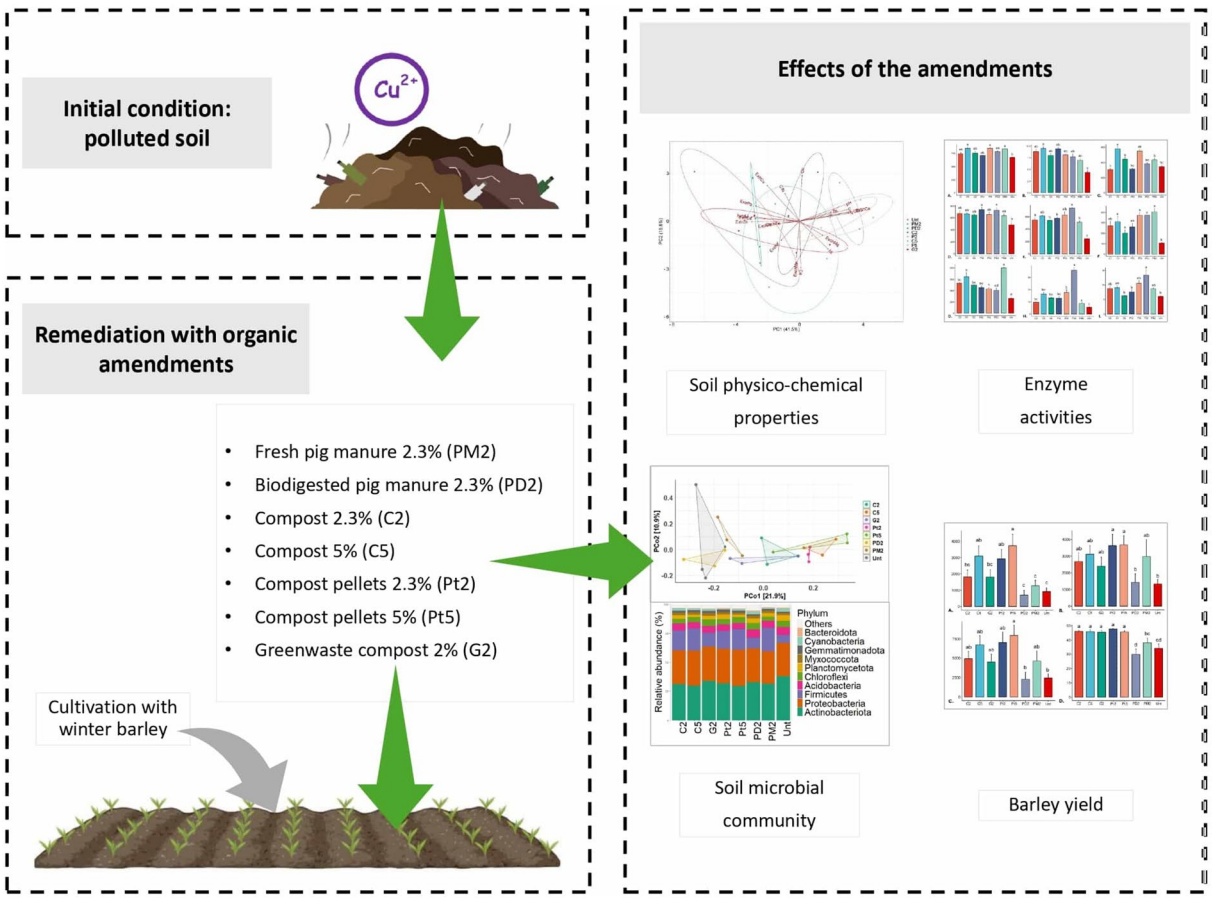
Keywords Soil contamination, Organic amendment, Metal, Microbial community, Soil fertility

Graphical Abstract



Background

Soil contamination with metal(loid)s and persistent organic pollutants (POPs) poses significant threats to soil health, compromises environmental quality, and creates pollutant pathways that finally endanger human health [1–4]. Anthropogenic activities, including agricultural, urban, and industrial processes, are primary sources of soil pollution. Among these, the wood processing industry has massively employed compounds such as Cu, Zn and Hg-based salts for wood preservation for decades. The uncontrolled release of chemicals is a significant driver of soil contamination, impairing soil fertility and ecological functionality [5, 6]. Thus, management approaches that reduce environmental pollution, and restore fertility and ecological functionality of contaminated soils are considered a sustainable option for managing large (peri)urban wasteland and brownfields, while also enabling their use for the production of non-food crops [7–12].

Current remediation strategies for metal(loid)-polluted soils include excavation, soil washing, and landfill disposal. Although these methods can effectively address small, localized contamination, they often lead to the irreversible removal or destruction of the soil, exacerbating environmental impacts in the affected areas [4]. These remediation approaches, focused on reducing pollutant linkages and associated risks to acceptable levels, are increasingly seen as unsustainable, particularly given the EU Soil Deal's priority of preserving soils as a slowly renewable natural resource, and its emphasis on adopting more sustainable management practices [11, 12]. Consequently, there is an urgent need for

sustainable approaches that restore soil health while minimizing environmental harm. Moreover, the growing demand of biomass for existing and new production processes is rising clean land-use conflicts between non-food and food crops. The possible use of contaminated soils for the cultivation of industrial/non-food crops offers both environmental benefits and new economic opportunities for site managers and farmers [13–15]. A promising alternative involves the combined use of appropriate plant species or plant assemblages along with soil microbes, eventually enhanced by organic and inorganic amendments. These amendments are key players in reacting with excess metal(loid)s through mechanisms, such as ion exchange, complexation, precipitation, and microbial interactions, thereby reducing pollutant mobility and bioavailability in the soil [16–19].

The cultivation of metal(loid)-excluding plants mitigates pollutants and allows the use of contaminated soils for either crop or biomass production [20–23]. Plants influence microbial growth and activity through rhizodeposition, accelerate the decomposition of soil organic matter (SOM), promote nutrient mineralization, and enhance humification [24–26]. SOM itself is pivotal in mitigating excessive metal exposure. Its components, such as humic and fulvic acids, possess high cation exchange capacity (CEC) and form chelate compounds with metal ions, reducing their bioavailability without altering their total soil concentrations [27–29].

The incorporation of soil amendments that decrease metal solubility further enhances immobilization processes [30–33]. A variety of low-cost organic materials and industrial by-products have been proposed as effective amendments. These include manure, fly ash, leaf mold, moss peat, green algae, biosolids, Fe/Mn compounds, such as iron grit and drinking water treatment sludge, and biochars. Various organic materials not only immobilize metals, but also improve the chemical and physical properties of soil by increasing SOM content, enhancing soil fertility, and reducing living organisms' exposure to pollutants and their toxicity [34–36]. In addition to immobilization, amendments enhance soil structure, pH, and nutrient availability, providing critical support for long-term soil health and productivity [37].

Despite the demonstrated efficacy of organic amendments in reducing pollutant bioavailability, limited research has examined their long-term impacts on microbial functionality and ecological restoration in highly contaminated soils. To address this gap, our study, conducted within the INTENSE (EU Era-Net Facce Surplus) project, evaluates the influence of various organic amendments incorporated into contaminated soils of field plots at a former wood preservation site after five years of phytomanagement. This work also focuses on present state of the contaminated field in comparison with the situation analysed at the beginning of phytomanagement. We analysed soil and microbial indicators—including soil physical and chemical properties, enzyme activities, bacterial abundance, and community composition—alongside with barley yield traits. We hypothesized that organic amendments reduce metal bioavailability while enhancing microbial activity and barley plant growth providing a sustainable remediation strategy.

Materials and methods

Experimental site

The site is a former wood preservation facility located in Saint-Médard d'Eyrans, Gironde, Southwestern France (N 44°43.35', W 00°30.94'), characterized by a temperate Atlantic climate. The site spans approximately 10 ha and includes natural attenuation areas with plant communities dominated by *Agrostis capillaris*, *Rumex acetosella*, *Senecio inaequidens*, *Populus nigra*, *Salix caprea*, and *Cytisus scoparius* [38].

Anthropogenic topsoils at the site have developed on an alluvial sandy soil classified as Fluvisol, Eutric Gleysols [39]. The soil texture is predominantly sandy (85.8% sand, 8.3% silt, and 5.9% clay) with a pH of 7, low organic matter content (1.6%), a C/N ratio of 17.2, and a low CEC (3.5 cmol kg^{-1}).

The site has sustained industrial activity since 1846, initially linked to the construction of a railway line [40]. Subsequently, the site was used for wood preservation, leading to heavy contamination of the topsoil. The wood preservation processes initially involved creosote treatment, followed by Cu-based compounds (1913–1980) and chromated copper arsenate (1980–2006). These activities resulted in Cu concentrations in the topsoil ranging from 65 to 1460 mg kg^{-1}, leading to Cu phytotoxicity [40]. Other metal(loid)s such as As (9.8 mg kg^{-1}), Zn (46 mg kg^{-1}), and Cr (23 mg kg^{-1}) were found at background levels [38, 40].

Polycyclic aromatic hydrocarbons (PAHs) were also detected in the soil, with a ΣPAH value of 21 mg kg^{-1} dry weight. This included fluoranthene (2.9 mg kg^{-1}), pyrene (2.8 mg kg^{-1}), and benzo[b]fluoranthene (3.4 mg kg^{-1}) [41]. However, individual PAH levels were below guideline values for residential use [42].

Experimental design

The field trial (around 49 m^2), labelled as sub-site F1 [38], comprised 25 randomized plots (1 m × 1 m, with a spacing of 20 cm between plots) was established in March 2017 [17]. Five organic amendments were evaluated: fresh pig manure (PM), biodigested pig manure (PD), compost (C), compost pellets (Pt) produced from spent

mushroom substrate, biogas digestate, and straw, and green waste compost (G) (Table S1). All amendments were applied at a 2.3% w/w rate (PM2, PD2, C2, Pt2, and G2), while compost (C) and compost pellets (Pt) were also applied at a higher 5% w/w rate (C5 and Pt5). Each amendment was applied in triplicate across the plots and four plots were left untreated as control treatment [17].

Since 2017, the plots were cultivated with a succession of crops, including spring barley, sunflower, tobacco, and broad beans (as a winter crop). In October 2020, winter barley (*Hordeum vulgare* L. cv. Séduction) was sown in all plots (15 cm distance between each row). To ensure adequate plant nutrition, all plots were fertilized with an inorganic N-P-K-(SO_3) fertilizer ((NF U 42-001, 15%−15%−15% + (5.5%), Amaltis, F-79206 Parthenay, France) at 40 kg N–NH_4NO_3, 40 kg P_2O_5, 40 kg K_2O, and 14.6 kg SO_3 per ha at three key growth stages: tillering, stem elongation, and flag leaf emergence of the winter barley, as detailed in Mench et al. [17].

Soil sampling and analyses

Topsoil (0–11 cm) samples were collected in October 2021 after the cultivation of winter barley using a sampling cylinder. Three samples per plot were collected and pooled to form a composite sample weighing 1.4 kg. The composite soil samples were split into subsamples: fresh soil samples were immediately sieved at 4 mm and then kept at 4 °C to measure microbiological and biochemical parameters; air-dried soil samples were sieved at 2 mm (nylon mesh) and then ground to 250 µm (agate mortar) when required, for determining main chemical physicochemical properties using standard methods [43]. Metal concentrations were analysed by firstly calcination of organic matter at 450 °C, followed by wet digestion in a mixture of HF and $HClO_4$, and a final recovery in HNO_3. Elemental quantification was done using inductively coupled plasma/atomic emission spectroscopy (ICP-AES, for Cu, Ni and Zn) and ICP-MS (Cd and Pb). Extractable soil metals (1 M NH_4NO_3-extractable) were determined following Gryschko et al., [44] and used as a proxy of available soil metals. Exchangeable bases were extracted by 0.1 N ammonium acetate (pH 7) and analysed according to Sharma et al. [45].

Soil microbial biomass was estimated by determining the content of adenosyne triphosphate with the method of Ciardi and Nannipieri [46], whereas the soil respiration rate was measured by the rate of CO_2 evolution determined with the titration method of Anderson and Domsch [47], using 20 g of soil placed in sealed glass jars for 3 d at 25 °C in the dark, in the presence of 1 M NaOH. After 7 days of incubation, the NaOH was added with 0.75 N $BaCl_2$ and phenolphthalein indicator, and then the solution was titrated against 0.1 M HCl. Enzyme activities were determined using standard methods outlined in the literature. Alkaline and acid phosphomonoesterase activities were measured following the method of Tabatabai and Bremner [48]. Arylesterase activity was quantified based on Zornoza et al. [49], and the β-glucosidase activity was determined according to Tabatabai [50]. Urease activity was measured following Nannipieri et al. [51], while protease activity was assessed using N-benzoyl-amide as substrate, according to Ladd and Butler [52].

Total DNA extraction and microbial analysis

Total DNA extraction and qPCR analysis

For DNA extraction, 250 mg of each homogenised soil sample was sampled and air-dried at room temperature for 48 h. Total soil DNA was extracted using the DNeasy PowerSoil Pro Kit (Qiagen, Germany), following the manufacturer's protocol. Purified nucleic acid concentrations were quantified using a Qubit 3.0 fluorometer (Thermo Fisher Scientific, California) with the Qubit 1 × dsDNA High Sensitivity Assay Kit (Thermo Fisher Scientific, California).

The abundance of the 16S bacterial DNA was analysed using quantitative PCR (qPCR) on a QuantStudio 12 K-Flex apparatus (Termo Fisher Scientifc, California). The 16S primers and reaction mix are described in Maretto et al. [53]. Gene copy numbers of the 16S rRNA gene were calculated using the Ct (Cycle threshold) value, following the method outlined by Zanardo et al. [54].

16S metabarcoding and bioinformartic analysis

For library preparation, the hypervariable regions (V2, V3, V4, V6-7, V8, and V9) of the 16 s rRNA gene were amplified using the 16S Ion Metagenomics Kit (Thermo Fisher Scientific, California). The amplification program included an initial denaturation at 95 °C for 10 min, followed by 25 cycles of 30 s at 95 °C, 30 s at the annealing temperature, and 20 s at 72 °C, concluding with a final 1-min hold at 72 °C. Post-PCR, libraries were cleaned and normalized to a concentration of 30 ng μ^{-1}. Barcode ligation was performed using the Ion XpressPlus Fragment Library Kit and the Ion Xpress Barcode Kit (Thermo Fisher Scientific, California). Each sample was ligated with a distinctive barcode. An additional amplification step was carried out with the following program: 5 min 95 °C, 7 cycles of 15 s 95 °C, 15 s 58 °C, and 1 min 70 °C, with final storage at 4 °C. Amplified libraries were quantified using the Qubit 3.0 Fluorometer with the 1 × dsDNA High Sensitivity Assay Kit (Thermo Fisher Scientific, California). Libraries were pooled to a final concentration of 100 pM and processed according to the manufacturer's instructions using the Ion 520 and Ion 530 Kit–OT2

400 bp (Thermo Fisher Scientific, California). Sequencing was conducted on the Ion GeneStudio S5 System (Thermo Fisher Scientific, California).

Raw sequencing reads were processed to remove primer pairs using the MetagenomicsPP plugin from ThermoFisher Plugin site [55]. Subsequent analysis was performed with QIIME2 v2021.4 [56]. The "qiime dada2 plugin" was employed to denoising and obtaining high-quality reads, which were clustered into amplicon sequencing variants (ASVs) using the SILVA SSU v138.1 database as a taxonomic reference [57]. ASVs occurring fewer than four times were excluded, and the data were normalized using total sum scaling (TSS) to ensure comparability of sequencing depth among samples.

Alpha diversity metrics, species composition, beta diversity and all statistical analysis for microbiota differences were carried out using the microeco package (v1.44.0) in R software [58]. Alpha diversity metric was calculated using Chao1, Shannon, and Simpson indexes, and the statistically significant differences were highlighted according to the ANOVA test ($p < 0.01$). Beta diversity was analysed through Principal Coordinate Analysis (PCoA) based on the Bray–Curtis distances, followed by Permutational MANOVA (PERMANOVA) to assess statistical significance in sample clustering ($p < 0.05$). Linear Discriminant Analysis Effect Size (LEfSe) with an LDA score threshold of 7.0 was performed to identify metagenomic biomarkers at the genus level [59], using the microeco package (v1.44.0) in R software. Moreover, to decipher the differences between soil treatments in the microbial communities, we performed a differential abundance analysis using the ANOVA test ($p < 0.05$), at phylum and genus level.

Finally, functional predictions of microbial communities were performed using FAPROTAX (Functional Annotation of Prokaryotic Taxa [60] with default parameters and TSS normalisation to infer ecological processes at the genus level), then statistically different functional groups between soil treatments and control were assessed with Kruskall–Wallis test ($p < 0.05$) using the microeco package (v1.44.0) in R software.

Plant yield traits

The maximum shoot length of barley plants was measured at three key growth stages: the VI leaf expansion stage, during stem elongation, and immediately prior to harvest in mid-June. At harvest, stems were carefully brushed to remove soil particles. For each row, straw and ears were separated, placed into paper bags, and oven-dried at 50 °C until a constant weight was achieved. Subsequently, the dry weight (DW) yields of ears, straw, and grains, was recorded. In addition, the thousand-grain weight (TGW) was determined to assess grain size.

Statistical analyses

The data are expressed as the mean ± standard deviation (SD) for three replicates in the case of amended plots and four replicates for untreated control plots.

An one-way analysis of variance (ANOVA) was conducted to determine significant differences among treatments. Differences were considered significant when $p < 0.01$ in the case of physico-chemical soil properties, enzyme activity, soil DNA quantification; and when $p < 0.05$ in the case of microbial diversity and yield traits. When ANOVA indicated significance, Fisher's Protected Least Significant Difference (PLSD) test was applied as a post hoc analysis to identify specific pairwise differences among groups. To ensure the robustness of the results, assumptions of normality and homoscedasticity were evaluated using the Shapiro–Wilk test and Levene's test, respectively. Nonparametric tests (e.g., Kruskal–Wallis) were applied when these assumptions were not met.

Correlation analyses were carried out to explore relationships between plant yield traits, soil physico-chemical parameters, and microbial diversity. These correlations were visualized using correlation heatmaps generated in R software with the SRplot package [61].

Results

Effects of organic amendments on soil physico-chemical properties and metal availability (Table 1)

The soil pH value ranged from 6.36 in the PM2 soils to 7.04 in the C5 soils, with significant differences observed in the C5, Pt2, and Pt5 soils relative to the PM2 ones. Soil CEC varied between 3.86 cmol kg^{-1} in the untreated (Unt) soils and 8.05 cmol kg^{-1} in the Pt2 soils; however, these differences were not statistically significant. The available phosphorus (Olsen-P) concentration ranged from 36 mg kg^{-1} in the Unt and C2 soils to 51 mg kg^{-1} in the Pt5 ones, with a significant 42% difference. Organic carbon (C) content (in g kg^{-1}) showed significant variation among treatments, ranging from 10.8 in the Unt soils to 20 in the C5 (+85%) and Pt5 soils. The total soil nitrogen (N) followed a similar trend, increasing significantly under Pt5 (+104%) and C5 (+103%) treatments compared to the Unt one, with values ranging from 0.76 g kg^{-1} (Unt) to 1.47 g kg^{-1} (Pt5). Despite these variations, the C/N ratio remained relatively stable across treatments, with values ranging narrowly from 13.45 to 14.37, with no significant differences.

Total soil concentrations of Co, Cu, Ni, and Zn did not significantly differ among treatments, and showed

Table 1 Selected chemical properties of topsoil (0–11 cm soil depth)

Soil parameters	Soil treatments							
	Unt	C2	C5	G2	Pt2	Pt5	PM2	PD2
pH water	6.50 ± 0.1 bc	6.73 ± 0.1 bc	7.04 ± 0.1 ab	6.60 ± 0.2 bc	6.96 ± 0.1 ab	6.92 ± 0.1 ab	6.36 ± 0.1 c	6.47 ± 0.02 bc
CEC ($cmol^+ kg^{-1}$)	3.86 ± 0.7 a	5.65 ± 1.6 a	7.29 ± 0.6 a	5.03 ± 1.6 a	8.05 ± 2.0 a	7.80 ± 1.5 a	5.36 ± 1.8 a	4.36 ± 0.2 a
P-Olsen (mg kg^{-1})	36.55 ± 1.9 b	36.35 ± 3.0 b	46.71 ± 2.3 ab	36.86 ± 6.6 ab	38.91 ± 4.5 ab	51.05 ± 6.7 a	42.30 ± 2.9 ab	41.10 ± 3.8 ab
Organic C (g kg^{-1})	10.80 ± 1.0 b	15.18 ± 5.2 ab	20.06 ± 1.7 a	14.56 ± 4.3 ab	17.89 ± 4.1 ab	20.01 ± 4.1 a	17.30 ± 4.3 ab	13.27 ± 2.1 ab
Total N (g kg^{-1})	0.76 ± 0.1 b	1.12 ± 0.3 ab	1.46 ± 0.1 a	1.04 ± 0.3 ab	1.29 ± 0.2 ab	1.47 ± 0.3 a	1.26 ± 0.3 ab	0.92 ± 0.1 ab
C/N	14.24 ± 0.5 a	13.45 ± 1.0 a	13.70 ± 0.2 a	13.96 ± 0.5 a	13.76 ± 0.6 a	13.57 ± 0.2 a	13.68 ± 0.8 a	14.37 ± 0.2 a
Total Co (mg kg^{-1})	1.58 ± 0.4 a	1.51 ± 0.2 a	1.67 ± 0.2 a	1.70 ± 0.2 a	1.71 ± 0.1 a	1.57 ± 0.1 a	1.94 ± 0.5 a	1.74 ± 0.3 a
Total Cu (mg kg^{-1})	956.27 ± 281.9 a	1061.13 ± 481.8 a	1174.90 ± 379.2 a	1002.57 ± 59.5 a	1110.70 ± 162.9 a	978.97 ± 247.5 a	845.10 ± 336.2 a	1191.03 ± 325.8 a
Total Ni (mg kg^{-1})	5.10 ± 0.7 a	4.99 ± 0.2 a	5.48 ± 0.5 a	5.22 ± 0.8 a	5.48 ± 0.1 a	5.79 ± 0.5 a	5.53 ± 0.7 a	5.28 ± 0.2 a
Total Zn (mg kg^{-1})	48.68 ± 7.3 a	60.85 ± 20.3 a	58.87 ± 6.3 a	56.11 ± 17.7 a	71.85 ± 13.8 a	66.15 ± 14.7 a	67.30 ± 19.2 a	56.25 ± 2.3 a
Extractable Cd (μg kg^{-1})	11.53 ± 5.4 a	5.06 ± 3.0 ab	1.83 ± 0.4 b	7.57 ± 0.5 ab	3.53 ± 0.9 ab	2.31 ± 0.1 b	7.17 ± 1.0 ab	9.06 ± 0.2 a
Extractable Cu (mg kg^{-1})	18.04 ± 17.7 a	6.10 ± 3.1 a	8.89 ± 5.2 a	8.45 ± 3.2 a	6.12 ± 0.9 a	7.12 ± 2.5 a	5.82 ± 3.8 a	15.13 ± 6.5 a
Extractable Ni (μg kg^{-1})	39.23 ± 21.8 ab	13.85 ± 4.1 ab	6.84 ± 2.5 b	28.38 ± 10.8 ab	7.41 ± 1.6 b	7.24 ± 3.5 b	38.87 ± 10.6 ab	45.14 ± 9.6 a
Extractable Pb (μg kg^{-1})	21.62 ± 13.6 ab	12.25 ± 12.9 ab	2.39 ± 0.2 b	17.37 ± 10.7 ab	7.73 ± 7.9 ab	3.29 ± 1.2 ab	16.33 ± 6.4 ab	22.64 ± 11.1 a
Extractable Zn (mg kg^{-1})	3.30 ± 1.8 a	1.09 ± 0.5 ab	0.25 ± 0.1 b	2.26 ± 1.1 ab	0.67 ± 0.4 ab	0.36 ± 0.2 b	3.13 ± 1.0 a	3.63 ± 0.3 a
Exchangeable Al ($mmol^+ kg^{-1}$)	3.36 ± 0.8 a	3.40 ± 0.74 a	1.96 ± 0.9 a	4.55 ± 3.7 a	2.80 ± 0.8 a	2.98 ± 0.8 a	3.07 ± 0.5 a	3.11 ± 0.8 a
Exchangeable Ca ($cmol^+ kg^{-1}$)	3.07 ± 0.7 a	4.97 ± 1.5 a	6.83 ± 0.4 a	4.20 ± 1.5 a	7.31 ± 1.9 a	7.33 ± 1.7 a	4.41 ± 1.8 a	3.47 ± 0.1 a
Exchangeable Fe ($\mu mol^+ kg^{-1}$)	4.73 ± 0.6 a	4.73 ± 1.3 a	< 5	7.07 ± 5.1 a	4.53 ± 0.9 a	4.6 ± 1.0 a	< 5	4.43 ± 0.7 a
Exchangeable K ($cmol^+ kg^{-1}$)	0.24 ± 0.03 a	0.19 ± 0.02 a	0.21 ± 0.01 a	0.26 ± 0.05 a	0.22 ± 0.05 a	0.20 ± 0.05 a	0.23 ± 0.02 a	0.23 ± 0.05 a
Exchangeable Mg ($cmol^+ kg^{-1}$)	0.02 ± 0.03 a	0.24 ± 0.05 a	0.26 ± 0.01 a	0.32 ± 0.1 a	0.30 ± 0.1 a	0.30 ± 0.04 a	0.40 ± 0.03 a	0.27 ± 0.02 a
Exchangeable Mn (mg kg^{-1})	7.90 ± 5.1 a	4.83 ± 0.8 a	4.67 ± 1.1 a	7.1 ± 2.8 a	6.90 ± 2.1 a	4.40 ± 0.7 a	9.47 ± 4.5 a	6.80 ± 3.8 a

Values are expressed as mean ± standard deviation ($n = 3$; Unt: $n = 4$); Unt: untreated soil; C2: 2.3% w/w of compost; C5: 5% w/w of compost; G2: 2.3% w/w of green waste compost; Pt2: 2.3% w/w of compost pellets; Pt5: 5% w/w of compost pellets; PD2: 2.3% w/w of digested pig manure; PM2: 2.3% w/w of pig manure. Different letters indicate statistically significant difference according to the ANOVA test ($p < 0.01$)

the following concentration ranges (in mg kg^{-1}): Co (1.51–1.94), Cu (845–1191 mg kg^{-1}), Ni (5.0–5.8), and Zn (48.7–71.8). Differently, the availability of Cd and Zn were significantly reduced in the C5 and Pt5 soils as compared to the Unt ones (− 84% and − 78% for Cd, − 93% and − 90% for Zn, respectively). Extractable Ni levels were significantly lower in the C5, Pt2, and Pt5 soils as compared to the PD2 soils (− 85%, − 84%, and − 84%, respectively). In addition, the C5 treatment significantly reduced extractable Pb concentrations as compared to the PD2 one (− 89%). No significant differences in extractable Cu concentrations were observed among

treatments. The amendments did not significantly affect the exchangeable bases (Al, Ca, Fe, K, Mg, and Mn).

Principal component analysis (PCA) revealed distinct clustering of C2, Pt2, C5, and Pt5 soils from the Unt ones, characterized by high loadings of organic C, CEC, exchangeable Ca, and extractable Cd, Zn, Ni, and Pb. PC1 accounted for 41.1% of the total variability (Figure S1).

Effects of organic amendments on soil physico-chemical properties and metal availability over a 5-year period

As part of the INTENSE (EU Era-Net Facce Surplus) project, the effects of soil amendments were initially evaluated in 2017 [17] and re-assessed in this study after a five-year period.

Compared to year 1, soil pH in year 5 showed a slight general decrease, most pronounced in the G2 soils (−11%). Soil CEC levels remained relatively stable, increasing by 29% and 31% in the Unt and PM2 soils, respectively, while decreasing by 22% in the PD2 soil over the five-year period. Available soil P (Olsen-P) remained unchanged in the Unt soils between years 1 and 5, whereas it decreased in all treated soils, ranging from −29% in G2 to −65% in C5. Soil organic C and total N contents were similar in year 5 compared to year 1 across all amended plots, while the Unt soils exhibited increases in organic C and total N (+50%) (Table S2).

The total concentrations of Co, Cu, Ni, and Zn remained stable in year 5 compared to year 1, with only minor fluctuations (Table S2). In contrast, extractable soil Cd greatly increased (+107% on average) in year 5 as compared to year 1 across all soils. Extractable soil Cu decreased by 52% on average in all amended soils, except for the PD2 one (+116%). Extractable soil Zn levels showed a marked increase across all amended soils after five years (almost + 800% on average) (Table S2).

Effect of organic amendments on soil microbial biomass, soil respiration and soil enzyme activity

Soil microbial biomass showed a significant increase in the C5 (+25%), Pt5 (+26%), and PM2 (+24%) soils compared to Unt (Fig. 1A). Soil respiration increased in all amended soil compared to Unt soil, with the highest increment found in the C5 soil (+116%) (Fig. 1B).

Activity of enzymes involved in C, P, and S mineralization was significanlty increased by the amendments. The C5 treatment significantly enhanced the activity of acid phosphomonoesterase (+67%) as compared to Unt. Alkaline phosphomonoesterase activity was significantly higher in the Pt2 and PD2 soils, increasing by 51.9% and 53.5%, respectively, compared to the Unt soils (Fig. 1C,D). All the amendments significantly stimulated β-glucosidase activity, with increases ranging from + 109% (PM2) and + 203% (PD2) compared to the Unt soils (Fig. 1E). Arylsulfatase activity was significantly increased by all treatments from 187% (C5) to 275% (PM2) (Fig. 1F). Similarly, arylesterase activity was significantly elevated in response to C2, C5, G2, Pt2, Pt5, and PM2 treatments, with increases ranging from + 62% (Pt5) to + 200% (PM2) (Fig. 1G).

The impact of the amendments on enzymes involved in N mineralization was less pronounced. Nevertheless, protease and urease activities were significantly increased by the PD2 treatment, showing remarkable enhancements of 547% and 120%, respectively. Additionally, protaese activity significantly peaked (+217%) in the Pt5 soils compared to the Unt ones (Fig. 1H,I).

Total soil DNA, microbial community dynamics and functional group in response to soil amendments

The total soil DNA content was significantly higher in Pt5 soils, showing a 452% increase as compared to all other soils, including the untreated (Unt) ones (Table S3). Similarly, qPCR analysis targeting the 16S rRNA gene revealed a 545% increase in bacterial gene copy numbers in Pt5 soils relative to Unt soils (Fig. 2).

Metabarcoding of the 16S rRNA gene yielded 6,152,607 reads, with an average read length of 229 bp and a mean of 236,638 ± 34,729 raw reads per sample. After quality filtering, 2,108,042 reads were retained and clustered into 81,656 amplicon sequence variants (ASVs). Taxonomic classification identified 26 phyla, 74 classes, 195 orders, 330 families, and 759 genera across all soil samples.

Alpha-diversity was assessed using the Chao1 (richness), Shannon (diversity), and Simpson (evenness) indexes. No significant differences were observed in the amended soils compared to the untreated (Unt) across the three indices (Fig. 3). As for differences among treated soils, Chao1 index was significantly higher in the C2 soils than in the G2 ones (Fig. 3A) and Shannon index was significantly enhanced in the Pt2 and C2 soils as compared with the PM2 soils (Fig. 3B). The Simpson index indicated high diversity across all soils, with most microbial communities being relatively even. However, PM2 soils showed significantly lower diversity, suggesting dominance by fewer species and reduced evenness. (Fig. 3C).

Principal coordinate analysis (PCoA) based on the ASV taxonomy highlighted significant differences in the structure of bacterial communities between the Unt soils and the C5, Pt2, and Pt5 soils (Fig. 4), as confirmed by PERMANOVA (Figure S5).

Relative abundance analysis evidenced that three phyla—*Actinobacteriota* (32%), *Proteobacteria* (29%) and *Firmicutes* (15%)—accounted for approximately 80% of the total bacterial community. Other phyla with notable

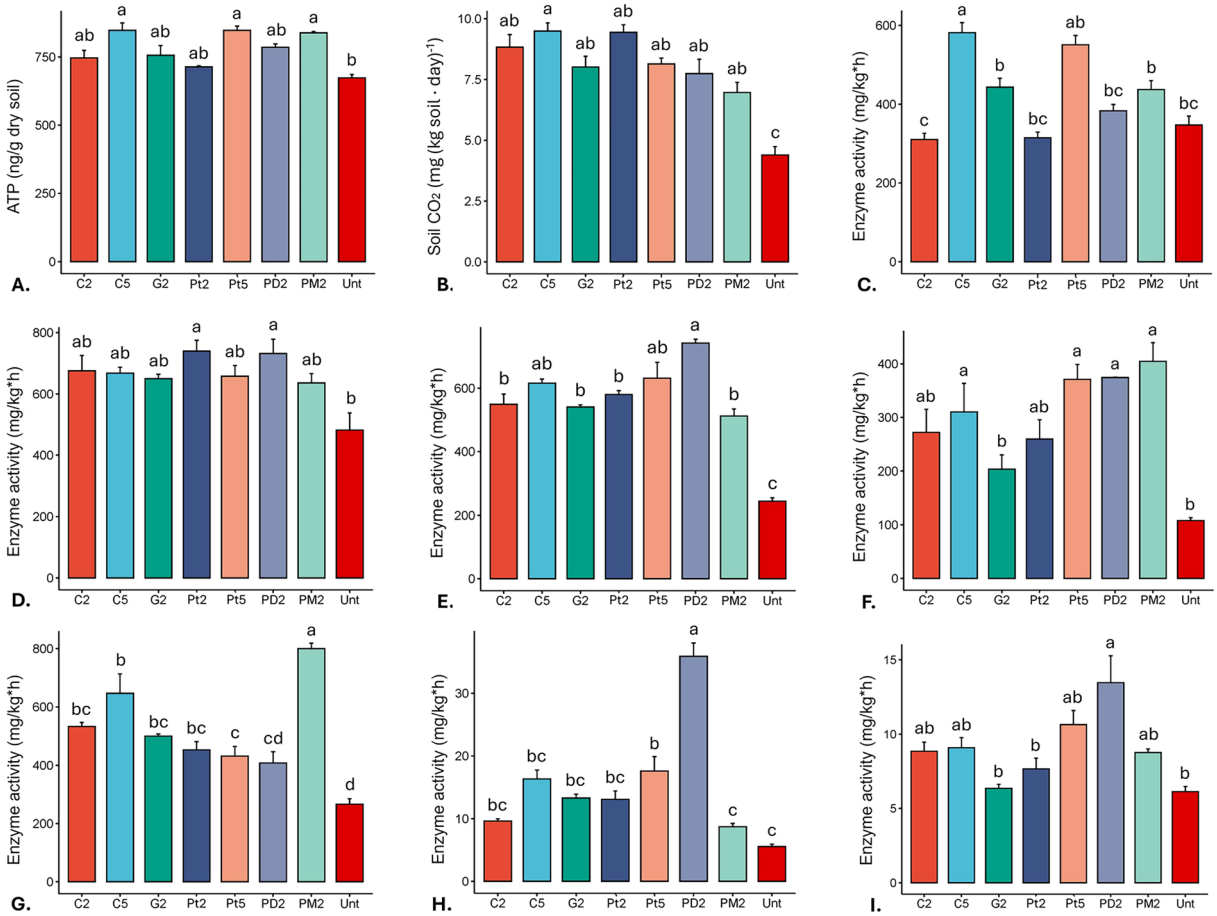


Fig. 1 Soil microbial biomass, soil respiration, and soil enzyme activity. Soil microbial biomass as estimated by ATP content (ng/g dry soil) (**A**). Soil respiration rate (mg CO_2-C kg^{-1} soil d^{-1}) (**B**). Soil enzyme activities involved in carbon, phosphorus, and sulfur mineralization: acid phosphomonoesterase (**C**); alkaline phosphomonoesterase (**D**); β-glucosidase (**E**); arylsulfatase (**F**); arylesterase (**G**). Soil enzyme activities involved in nitrogen mineralization: protease (**H**); urease (**I**). Enzyme activities are expressed as mg kg^{-1} h^{-1}. Different letters indicate statistically significant differences according to the ANOVA test ($p < 0.01$)

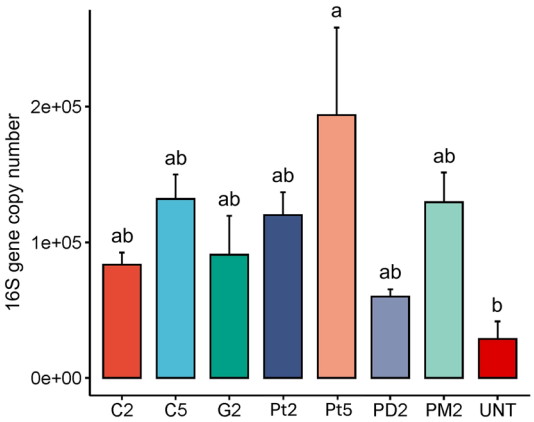


Fig. 2. 16S gene copies number as resulting from qPCR analysis. Different letters indicate statistically significant difference according to the ANOVA test ($p < 0.01$)

abundance included *Acidobacteria* (6%), *Chloroflexi* (4.8%) and *Planctomycetoa* (3.1%) (Fig. 5A). *Actinobacteriota* were significantly more abundant in the Unt soils as compared to the C2, C5, Pt2, Pt5, PM2, and PD2 ones, while *Firmicutes* were significantly more abundant in the C2, C5, Pt2, Pt5, and PM2-soils than in the Unt soils. Differences in the abundance of other phyla were not statistically significant (Table S4).

At genus level, the most abundant taxa included *Bacillus* (4.3%), *Streptomyces* (4%), *Bradyrhizobium* (3.7%), *Sphingominas* (3.2%), *MND1* (3.2%), *67–14* (2.8%), *Acidibacter* (2.1%), *Nocardioides* (2%), *KD4-96* (2%), and *Blastococcus* (1.5%) (Fig. 5B). Among these, *Bacillus* was significantly more abundant in the Pt5 soils compared to the PM2 ones, while *MND1* was significantly more abundant in the Pt5, Pt2, G2, and C5 soils than in the Unt soils. Other genera did not show significant changes (Table S4).

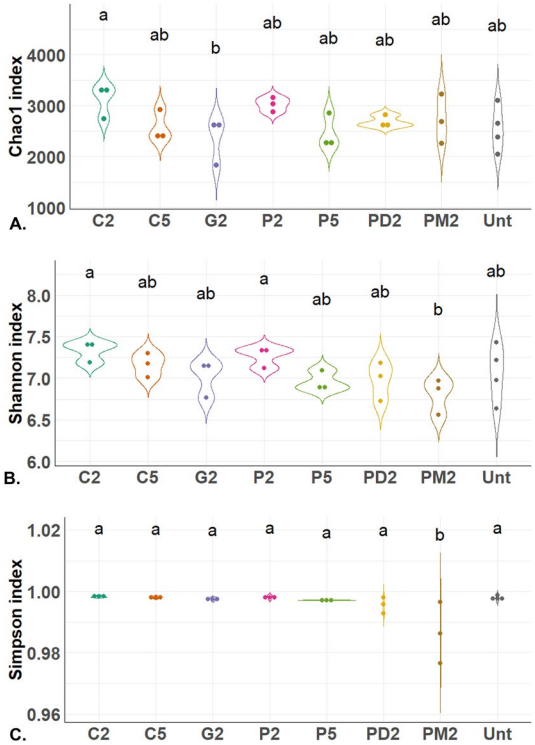


Fig. 3 Soil microbial diversity as described by Chao1 (**A**), Shannon (**B**), and Simpson (**C**) index. Box plots of alpha diversity values distribution for the different conditions. The line inside each box represents the median value. The outliers are shown as dots. Different letters indicate statistically significant difference according to the ANOVA test ($p < 0.05$)

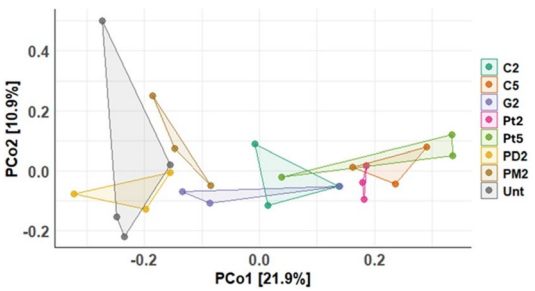


Fig. 4 Principal coordinate analysis (PCoA) of bacterial community compositions at OTUs level based on the Bray–Curtis dissimilarity matrix across differently amended soils. Shapes are color-coded by treatment groups

Linear discriminant analysis (LDA) identified genus-level biomarkers specific to each treatment. The treatments with the highest number of biomarker microbial groups were C2 and Pt5 (Fig. 6). Key biomarker groups for each treatment were *Paenibacillus*, *Lysinibacillus*, and *Thermobacillus* for C2; *IS-44*, *Agromyces*, *Desulfofarcimen* for C5; *S0134* and *Laceyella* for G2; *67–14*, *Vicinamibacteraceae* and *Rhodomicrobium* for Pt2; *Limnochordaceae*, *Ureibacillus*, and *CCD24* for Pt5; *Clostridium*, *Candidatus Solibacter* and *Turicibacter* for PD2; *Planifilum*, *Oceanobacillus*, and *Saccharopolyspora* for PM2; and *Mycobacterium*, *Conexibacter* and *Jatrophihabitans* for Unt. Among these genera, *Paenibacillus, Lysinibacillus,* and *Ureibacillus* were significantly more abundant in the Pt5, Pt2, C5, and C2 soils than in the Unt soils. Conversely, *Mycobacterium*, *Conexibacter* and *Jatrophihabitans* were significantly less abundant in all amended soils as compared to the Unt ones (Table S4).

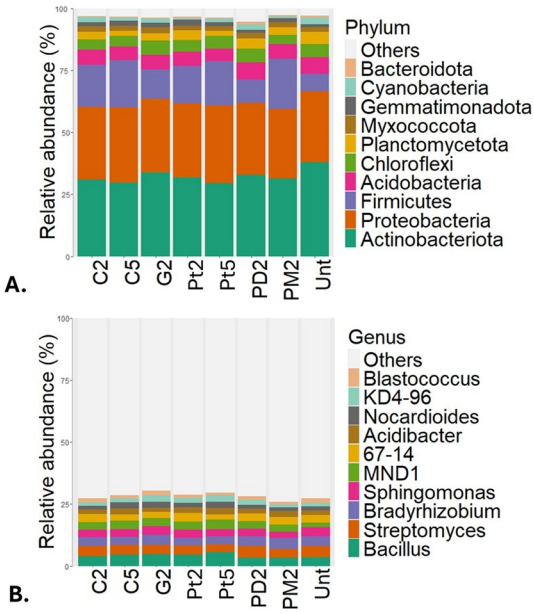


Fig. 5 Relative abundances (%) of the dominant bacteria across different treatments, at the phylum level (**A**), at the genus level (**B**)

According to FAPROTAX functional annotations, dominant bacterial functional groups were associated with chemoheterotrophy, and aerobic chemoheterotrophy (Fig. 7A). Aerobic chemoheterotrophy processes were significantly lower in the C5, Pt2, and Pt5 soils than in the Unt ones (Fig. 7A). Other dominant bacterial functional groups were associated with nitrification and aerobic ammonia oxidation (Fig. 7B). These processes were significantly promoted in the C2, C5, and Pt5 soils compared to the Unt ones (Fig. 7B).

Plant yield in response to soil amendments

The dry weight (DW) grain yield of winter barley increased significantly in the C5, Pt2, and Pt5 plots as compared to the Unt plots, with increases of by 239%, 219%, and 307%, respectively (Fig. 8A). Similarly, the straw DW yield of winter barley was significantly higher in the Pt2 and Pt5 plots than in the Unt plots, with

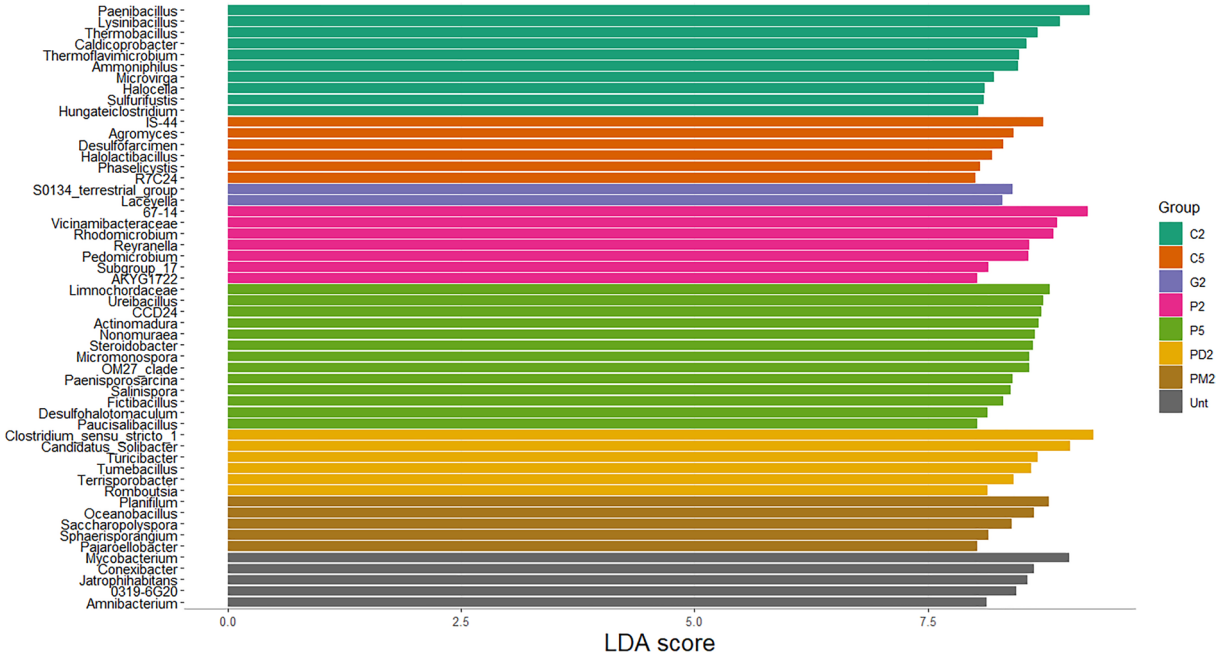


Fig. 6 LEfSe analysis highlighting the different genera biomarkers among the eight groups (LDA score threshold of 7.0 was set for discriminative features)

increases of 172% and 176%, respectively (Fig. 8B). Furthermore, the total shoot DW yield of barley from the Pt5 plots was significantly higher (+225%) than that from the Unt plots (Fig. 8C). The one-thousand grain DW yield significantly increased under the C2, Pt2, C5, Pt5, and G2 treatments as compared to the Unt one, with an average increase of 34% (Fig. 8D). Overall, the 5% compost application rate exerted a sustained positive effect on winter barley growth during the fifth year of the trial.

Pearson's correlation analyses

Pearson's correlation coefficients were calculated to assess relationships among soil properties (pH, CEC, available P, organic C, total N, and extractable metals—Cd, Cu, Ni, Pb, and Zn), microbial communities, enzyme activities, and barley yield traits (grain, straw, ear, and 1000-grain DW yields). Given such correlations, enzyme activities involved in C, P, and S mineralization significantly correlated positively with soil CEC, available P (Olsen-P), total N and organic C contents. The strongest correlation occurred between acid phosphomonoesterase activity and available P (R = 0.84) (Fig. 9A). Conversely, extractable soil metals negatively affected enzyme activities. In particular, 1 M NH_4NO_3-extractable soil Cu had the strongest negative correlation with arylesterase (R = − 0.67). Enzyme activities involved in N mineralization (i.e. urease and protease) did not display significant correlation with soil properties (Fig. 9A).

Correlations were also analysed between the most abundant biomarker genera identified through LEfSe, yield traits (grain, straw, ear, and 1000-grain DW yields), and soil properties (pH, CEC, available P, organic C, total N, and extractable metals—Cd, Cu, Ni, Pb, and Zn) (Fig. 9B). Accordingly, bacterial genera positively correlated with yields were also positively correlated with beneficial soil properties (higher pH, CEC, available P, organic C, and total N) and negatively correlated with extractable soil metals. Conversely, bacterial taxa negatively correlated with yields and beneficial soil features showed positive correlations with extractable soil metals.

Biomarker groups specific to the C2 (*Paenibacillus*, *Lysinibacillus*, and *Thermobacillus*), C5 (*IS-44*, *Agromyces*, and *Desulfofarcimen*), Pt2 (*67-14*, *Vicinamibacteraceae*, and *Rhodomicrobium*), and Pt5 soils (*Limnochordaceae*, *Ureibacillus*, and *CCD24*) positively correlated with yields, soil pH value, soil CEC, organic C, total N, and available P and negatively correlated with extractable metals ($p < 0.01$) (Fig. 9B). Conversely, biomarker groups specific to the G2 soils (*S0134* and *Laceyella*) and PM2 soils (*Planifilum*, *Oceanobacillus*, and *Saccharopolyspora*) displayed

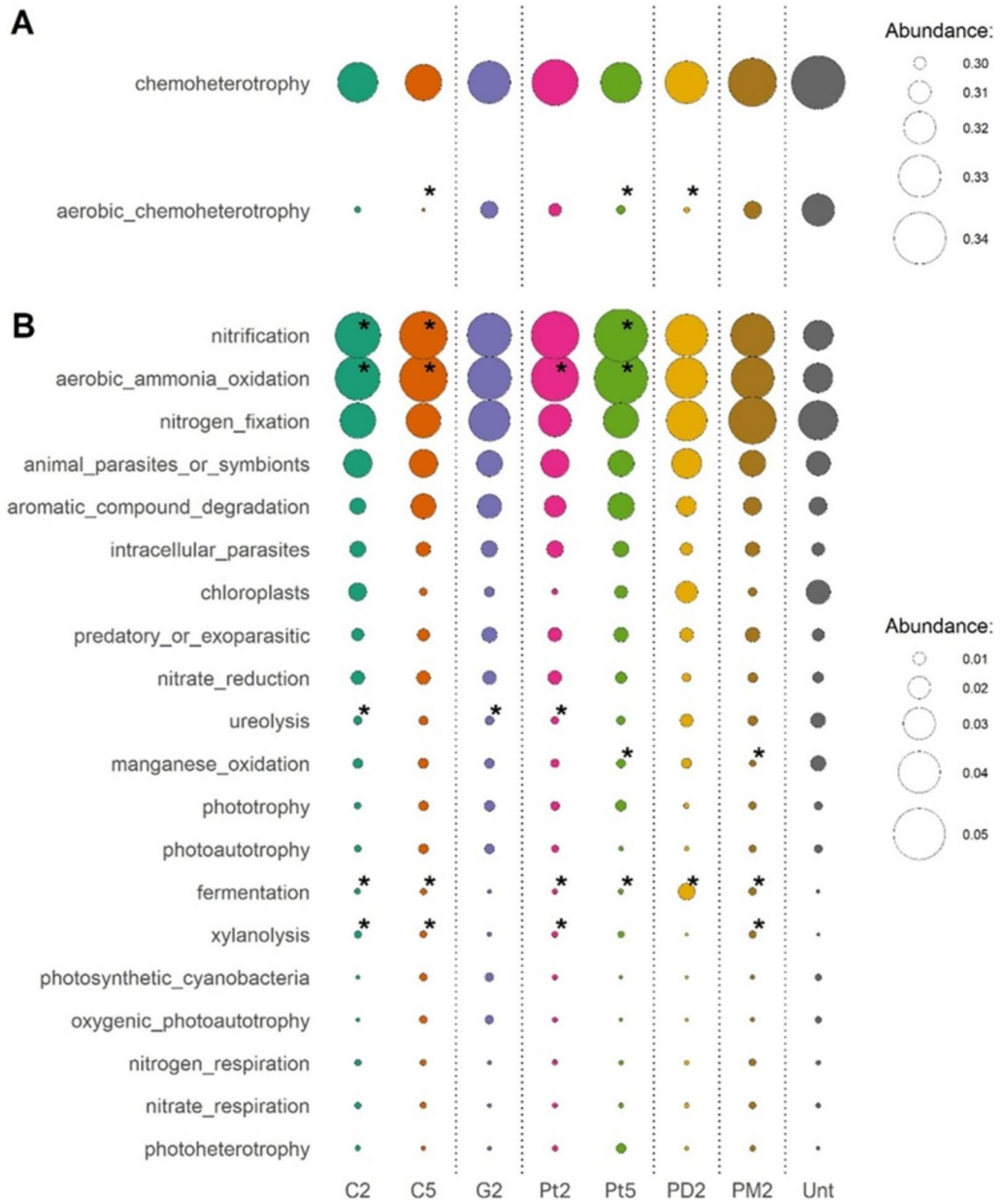


Fig. 7 Bubble plot of the most abundant functional groups of bacteria in the soil treatments. Most represented functional groups (**A**) are separated from less represented functional groups (**B**) for graphical reasons. * represents statistically different functional groups between soil treatments and control (Kruskall–Wallis test, $p < 0.05$)

weak and inconsistent correlations with soil parameters (Fig. 9B). Finally, biomarker groups from the PD2 soils (*Clostridium*, *Candidatus Solibacter*, and *Turicibacter*) and Unt soils (*Mycobacterium*, *Conexibacter*, and *Jatrophihabitans*) were negatively correlated with yields and soil properties but positively correlated with extractable metals (Fig. 9B).

Discussion

Soil represents a reservoir of nutrients necessary for crop development, with its physical and chemical properties being pivotal in determining plant growth and productivity. Soil pH, nutrient availability, and organic matter content are key indicators of soil fertility and its capacity to sustain crop production.

Here, soil properties of field plots initially measured in 2017 [17] were re-evaluated five years later to monitor

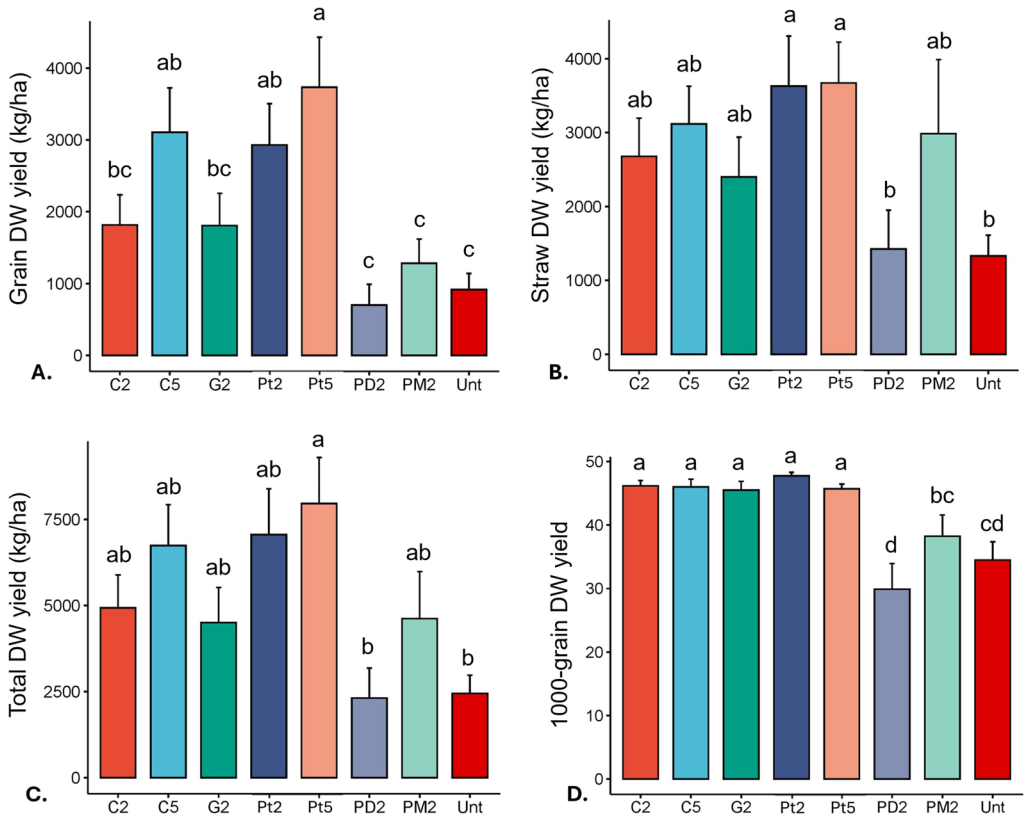


Fig. 8 Winter barley yield traits. Grain dry weight (DW) yield (**A**), straw DW yield (**B**), total DW yield (**C**) and one-thousand grain DW yield (**D**) of winter barley plants in kg/ha, grown in the field plots. Different letters indicate statistically significant difference according to the ANOVA test ($p < 0.05$)

(See figure on next page.)

Fig. 9 Correlation plots. The activity of the enzymes acid and alkaline phosphomonoesterase, arylesterase, B-glucosidase**,** arylsulfatase, protease, and urease, were correlated with selected soil properties (pH, CEC, available P, organic C, total N), and extractable Cu, Zn, Cd, Ni, and Pb (**A**). Yield traits (grain, straw, ear, and 1000-grain dry weight), selected soil properties (pH, CEC, available P, organic C, total N), and extractable Cu, Zn, Cd, Cr, Ni, and Pb, were correlated with the three most abundant biomarkers detected by LEfSe analysis for each treatment. Along the top, the three categories of parameters (yield traits, soil properties, and enzyme activities) are listed. On the left, the treatments with corresponding biomarkers (C2, C5, G2, P2, P5, PD2, PM2, and Unt) are shown. Treatments with biomarkers positively correlated with yield are highlighted in green (C2, C5, P2, and P5), while those negatively correlated are highlighted in red (PD2 and Unt) (**B**). Each coloured box displays Pearson's correlation coefficient for a pair of compounds. Red colour shapes indicate negative correlations, blue colour shapes indicate positive correlations, and the R value for each comparison is shown ($p < 0.01$, Pearson index)

the effect of the amendments on soil physico-chemical properties. The comparison of key soil properties between year 1 and the present evaluation showed a slight acidification of all soils, although the values were consistent with those observed in year 1. The acidic pH (5.8) of the PM2 soil reflected the inherent acidity of the pig manure, which contained less calcium compared to compost (C) and compost pellets (Pt) [17]. Despite the amendments, soil pH remained slightly lower than that of the same uncontaminated French fluvisol series, which averages around 7.4 [62]; dolomite addition could therefore be considered to counteract the gradual topsoil acidification. The stability of CEC over the time indicated that the treatments effectively preserved soil CEC, as previously reported for soils of the same French fluvisol series [62]. The decline in available soil P (Olsen-P) across all treatments was likely attributable to crop uptake, given that $H_2PO_4^-$, the bioavailable form of phosphorus, is actively utilized by plants. This interpretation is supported by the strong positive correlation between available P content and acid phosphomonoesterase activity (Fig. 9A). Furthermore, while P is typically considered

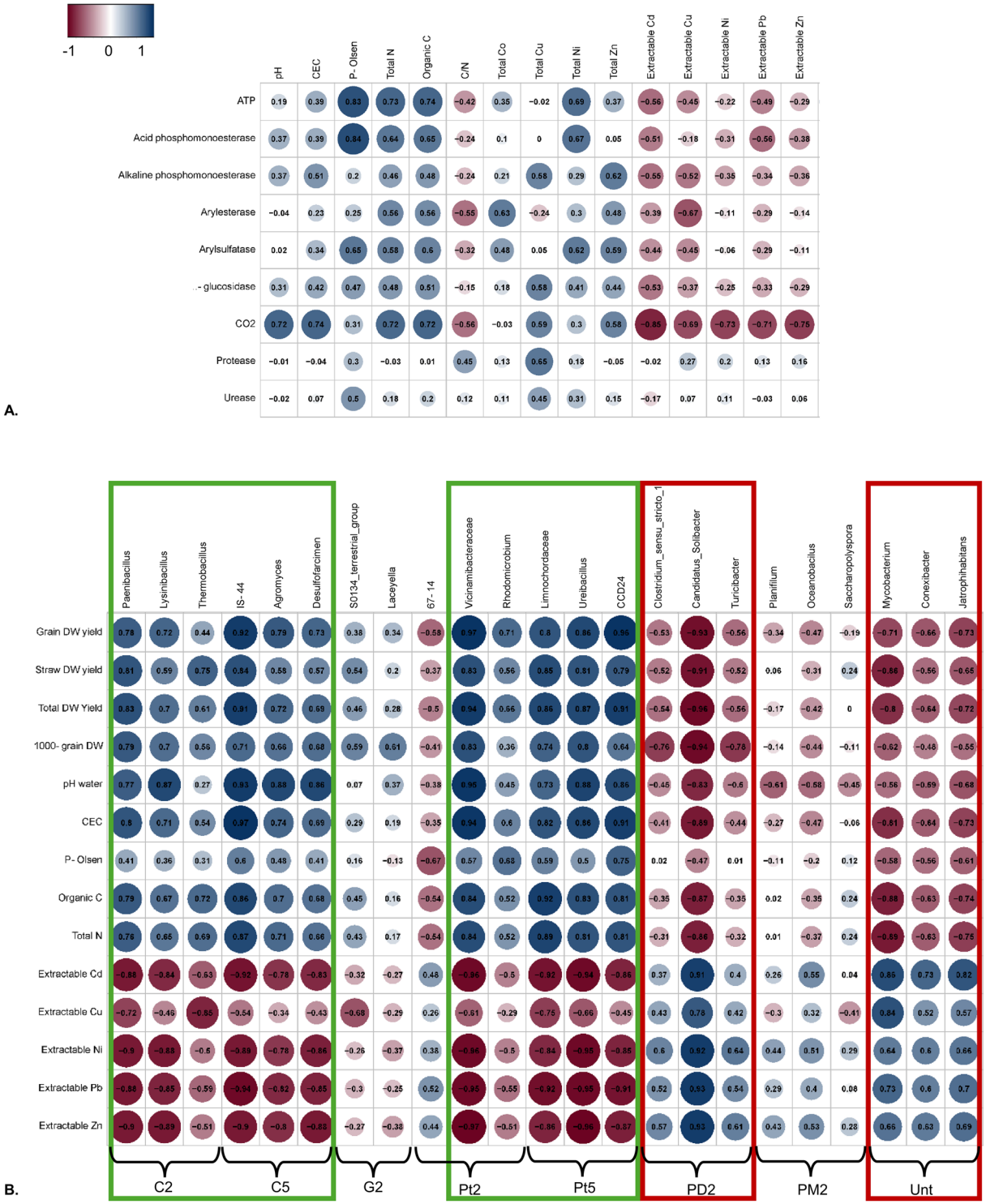


Fig. 9 (See legend on previous page.)

an element with low mobility, the sandy texture soil (> 80) [17] may have contributed to P leaching over time. The increase in soil organic C and total N in the Unt soils suggests that the initial content of these elements was very low and that recultivation likely enhanced soil C and N through rhizodeposition and root residue incorporation. Additionally, cereals such as barley are known to produce relatively high amounts of phytoliths, playing a key role

in carbon sequestration, particularly in poor, sandy soils [63]. Organic C and total N levels in the amended soils reached values typical of uncontaminated French sandy soils [40, 64, 65] and French fluvisol [62]. Notably, the C/N ratio remained satisfying in both year 1 and year 5, suggesting limited mineralization tendencies in the amended soils (C/N > 10) [66]. Overall, these results indicated a permanent increase of soil chemical fertility.

Similarly to year 1, total soil Cu largely exceeded the background values for French sandy soils (3.2–8.4 mg kg^{-1}) in all plots [17, 40, 64, 65]. Total soil Ni remained within background values for French sandy soils [40, 64, 65]. Total soil Zn remained relatively low, slightly exceeding the background values for French sandy soils (max 48 mg kg^{-1}) in all the amended plots but remained within the range in the untreated plots [17, 40, 64, 65].

The increase in extractable soil Cd is likely explained by the slight soil acidification over time. Notably, the lowest increase was observed in the C5 and Pt5 soils, which maintained neutral pH levels, thereby keeping extractable Cd consistently lower than in the Unt soils after five years [17]. Additional Cd inputs over time from the applied NPK fertilizer cannot be excluded. Its Cd concentration inherited from phosphate production is unknown. However, since July 22, 2022, the European Union has banned all inorganic fertilizers with a cadmium content in excess of 60 mg Cd kg^{-1}. Based on this maximum permitted concentration, our fertilization rate and a soil mass of 3000 Mg ha^{-1} (topsoil 0–30 cm), the potential increase in soil Cd would be up to 0.0024 mg kg^{-1} $year^{-1}$ (without considering potential removals through plant uptake and leaching). Total Cu concentrations remained stable across all plots between year 1 and year 5, but extractable soil Cu decreased, following a quadratic curve related to soil pH (Figure S2), a trend often reported in the literature. The well-known sorption of Cu by organic matter may also have played a role [17]. Extractable soil Zn increased substantially in all soils, likely driven by the slight acidification observed over time, similar to the trend seen for Cd [17]. Overall, Pt5 and C5 were effective in diminishing extractable Cd, Ni, Pb and Zn in year 5. These findings highlight the capacity of organic amendments, notably such compost at 5% w/w addition rate, to mitigate the effects of certain metals, considering a reduced extractable fraction, while contributing to improved soil quality over time. Organic amendments contribute to the in situ immobilization of heavy metals in soil by altering their chemical forms and reducing their mobility and bioavailability. This is achieved through several mechanisms, including complexation with functional groups, pH increase that promotes metal precipitation, and enhanced cation exchange capacity that improves metal adsorption [67]. Similar results have been reported in long-term studies showing that compost-based amendments significantly reduce the extractable fraction of heavy metals by promoting their stabilization in less bioavailable forms [68, 69].

To further evaluate the impact of soil amendments, key soil biochemical parameters were assessed at year 5, providing insights into microbial activity, nutrient cycling, and overall soil health.

The observed increase in microbial biomass and soil respiration across all treatments indicates a positive impact of soil amendments on the microbial ecosystem in metal-contaminated soils. Microbial biomass, a sensitive indicator of soil health under metal stress, increased suggesting improved conditions for microbial growth [70]. The concurrent rise in soil respiration reflects enhanced microbial metabolic activity. These findings demonstrate the efficacy of soil amendments in alleviating microbial stress in contaminated soils [71]. The improvements are likely attributable to reduced metal bioavailability (e.g. extractable soil Cu), nutrient supplementation, and enhanced soil physical properties, implying that amendments mitigated excessive exposure to metals inducing toxicity and promoted microbial vitality.

Changes in soil enzyme activity were analysed as indicators of potential improvements in soil quality and functionality. The increased activity of acid phosphomonoesterase in the C5 and Pt5 soils can be attributed to the P content in these amendments, the highest (6.3 g kg^{-1} DW) among the tested amendments (Table S1). It is well-documented that elevated levels of N, P, and C in the soil enhance phosphatase activity, particularly acid phosphomonoesterase [72]. This is consistent with the positive correlation between acid phosphomonoesterase activity and soil P, C, and N contents (Fig. 9A). For the Pt5 soils, although the amendment provided similar amounts of C, N, and P as C5, acid phosphomonoesterase activity was lower than in the C5 soils as pellets split and decomposed more slowly (some particles remaining visible). Although not statistically different from the Unt soil, this activity did not significantly differ from that of the C5 soils (Fig. 1C). Alkaline phosphomonoesterase activity, in contrast, was strongly inhibited by elevated levels of extractable soil Cu (Fig. 9A). This finding aligns with the previous research demonstrating the inhibitory effects of Cu on this activity [73]. Arylsulfatase activity was negatively correlated with S content in the amendments, although this correlation was weak [17]. This finding agreed with studies showing that arylsulfatase production is stimulated in soils where S is a limiting factor, as microorganisms respond to nutrient deficiencies by increasing enzyme synthesis.

Overall, the activity of all tested enzymes was positively influenced by key soil properties such as higher pH, CEC, available P, and organic C and N contents. Conversely, enzyme activity was negatively affected by elevated levels of extractable soil Cu (Fig. 9A). These results emphasize the role of enzymes as sensitive indicators of soil health, reflecting the microbial processes that drive nutrient cycling and availability. The enhancement of enzyme activity across amended plots highlights the beneficial effects of organic amendments on microbial functioning and nutrient cycling.

No significant differences in soil bacterial abundance were evidenced between amended and unamended plots (Fig. 3). This result was unexpected, as organic amendments are generally associated with increased soil taxonomic and functional diversity [74]. Interestingly, soil bacterial abundance and 16S rRNA gene copy numbers showed opposite behaviour. Nutrient-rich amendments (C5 and Pt5) led to reduced bacterial abundance but the highest 16S gene copy numbers (Figs. 2, 3), while unamended plots exhibited a reverse behaviour, confirming what showed by soil microbial biomass analysis (Fig. 1A). This pattern may reflect the improved physico-chemical properties associated with C5 and Pt5 treatments, which likely favour the proliferation of specific microbial populations [75]. Conversely, the harsher conditions in unamended plots may increase selection pressure, promoting greater microbial diversity but reducing total bacterial counts [76].

Unamended plots also exhibited higher variability among replicates, likely due to their inherent heterogeneity, whereas amended plots were more homogeneous. Notably, the C5 and Pt5 treatments, which contained the highest C content, stimulated significant vegetation growth, potentially contributing to the elevated 16S bacterial DNA determined in these soils. PCoA analysis further supported these findings, as C5 and Pt5 treatments, which had lower microbial diversity than Pt2 and C2, were more distinct from unamended plots (Fig. 4).

Actinobacteriota and *Proteobacteria* emerged as the most abundant phyla across all soil samples (Fig. 5A), which is consistent with the previous studies [75, 77, 78]. *Actinobacteriota* are known to dominate soil microbiota and are highly adaptable to extreme conditions [79]. Their prevalence in the Unt soils likely reflects their ability to thrive in harsh, nutrient-poor environments. *Proteobacteria*, also prominent across all soil samples (Fig. 5A), are key players in C, N, and S cycles and are well-adapted to nutrient-rich or contaminated conditions. Their tolerance to high metal concentrations and ability to utilize diverse carbon and nitrogen sources likely explains their abundance in these soils [78].

At the genus level, *Bacillus*, *Streptomyces* and *Bradyrhizobium* were the most abundant across soil samples (Fig. 5B). *Bacillus*, a plant growth-promoting microbe, contributes to P solubilization and shows potential for metal bioremediation [74, 78, 80]. Its role in enhancing plant growth in Cu-contaminated soils [81] highlights its applicability in bioremediation strategies for toxic environments [82]. *Streptomyces,* recognized for improving soil fertility and nutrient availability [74, 83, 84], displayed reduced abundance in amended plots. This decrease may result from competition with faster-growing copiotrophic bacteria in nutrient-rich conditions, as suggested by Miralles et al. [77]. Over time, as easily decomposable organic matter is depleted, *Streptomyces* may re-emerge due to their ability to degrade recalcitrant substrates and produce antibiotics [85]. Further studies with additional data points are needed to confirm this hypothesis. *Bradyrhizobium*, typically associated with N fixation in legumes [86], was unexpectedly detected. Its presence could be linked to past wood industry activities, as it may have played a role in wood decay [87], to broad bean crops (*Vicia faba*) prior to barley cultivation, and patches of *Cytisus scoparius* and *Lotus corniculatus* present before the experiment setup.

The LEfSe analysis identified amendments that were most effective and beneficial based on the biomarkers detected and their correlations with various soil parameters (Fig. 6). Bacterial genera biomarkers associated with the soils amended by compost and compost pellets showed consistent positive correlations with crop yield and favourable soil properties, while displaying negative correlations with extractable metals. In contrast, biomarkers from the G2, PD2, and PM2 soils exhibited weaker correlations with these parameters. An exception was *Candidatus Solibacter* (PD2), displaying a strong negative correlation with yield (grain, straw, total, and one thousand grain DW) and positive soil properties, alongside a positive correlation with extractable metals (Cd, Cu, Ni, Pb, Zn). Biomarkers identified in the Unt soils were negatively correlated with yield and beneficial soil properties, while positively correlated with extractable metals.

Paenibacillus and *Lysinibacillus* emerged as signature genera in the C2 soils. *Paenibacillus*, a key plant growth-promoting rhizobacteria (PGPR), is notable for its N fixation capabilities. It promotes plant growth through P solubilization, production of indole-3-acetic acid (IAA), and secretion of siderophores that enhance iron acquisition [88, 89]. Additionally, *P. polymyxa* produces hydrolytic enzymes, cell-wall-degrading enzymes, and antimicrobial compounds, including lipopeptides, fusaricidins, and polymyxins, contributing significantly to plant health and protection [90]. Species within the

Lysinibacillus genus have shown significant potential for metal remediation and are gaining attention as plant growth-promoting agents and biocontrol alternatives to agrochemicals [91, 92].

The genera *IS-44*, *Agromyces*, and *Desulfofarcimen* evidenced in the C5 soils also represent biomarkers of healthy soils. Notably, *Agromyces* has been reported for its dual role as a plant-growth promoter and an indicator of soil health [93, 94].

In the Pt2 soil, the genus *67-14* displayed contrasting behaviour, being typically associated with environments low in total organic carbon (TOC) and primarily linked to autotrophic carbon dioxide fixation [95], while *Vicinamibacteraceae*, first described in 2018 [96], was identified as an indicator of xenobiotic compound degradation and a contributor to carbon transformation processes [97, 98].

The genera *Limnochordaceae* and *Ureibacillus*, detected in the Pt5 soils, are associated with cellulose degradation. Both have been reported for their roles in cellulose enzymatic hydrolysis and humification, as well lignin degradation [99, 100].

Limited information is available regarding the agricultural roles of biomarkers found in the Unt soils. For instance, *Mycobacterium* is widely recognized for its association with human and animal diseases [101], while *Conexibacter* has been reported as particularly abundant in metal-polluted soils [102].

The functional profiling of soil microbial communities using FAPROTAX provided insight into the ecological processes potentially influenced by organic amendments. Chemoheterotrophy and aerobic chemoheterotrophy were the most prevalent functional groups, consistent with microbial communities in carbon-rich environments [103]. Interestingly, these functions were significantly reduced in the C5, Pt2, and Pt5 soils compared to the untreated (Unt) control, suggesting a shift from generalized carbon degradation towards more specialized functions. Indeed, processes related to nitrification and aerobic ammonia oxidation were significantly enhanced in the C2, C5, and Pt5 soils, likely reflecting increased nitrogen availability and a more active nitrogen cycle due to compost input. These results align with previous studies reporting that organic amendments can restructure microbial communities towards functions linked with nutrient cycling and plant growth promotion [104, 105]. The observed functional shifts further support the role of compost-based amendments in improving soil fertility through enhanced microbial activity and functional diversity, particularly under metal stress conditions.

Overall, the Pt5 and C5 treatments created the most favourable conditions for soil health, characterized by a high abundance of genera positively correlated with barley yield, nutrient-rich soil properties (C, N, and P contents), and reduced metal extractability. These treatments particularly enriched biomarkers associated with cellulose degradation and soil health promotion. From a microbiome perspective, the least effective treatment in fostering a microbial community associated with yield and soil quality was PD2, as it showed weaker correlations with beneficial soil parameters and a stronger association with extractable metals.

Barley plants cultivated at the site, both spring and winter varieties, exhibited limited heavy metal uptake, particularly for Cu, consistent with a metal-excluder behavior reported in cereals under similar conditions [106, 107]. This suggests a low risk of metal accumulation in grain, supporting the safe use of crops produced on the treated plots. The treatments with the raw compost and compost pellets were effective in increasing winter barley yield, especially when supply at the 5% addition rate (Fig. 8, Supplementary Table 2). Only barley grown on the PD2 plots showed a decrease in the total DW yield. In year 5, straw DW yield was approximately 50% lower in Pt5 and C5 (winter barley) as compared to year 1 (spring barley), but 60% higher, on average, in Pt2 and C2 over the same period [17]. Nonetheless, the 5% addition rate and pressing of compost (C5 and Pt5) showed a sustained positive effect, delivering the highest yields by year 5 despite a gradual decrease over time.

Conclusions

This study assessed the effects of various organic amendments and phytomanagement on health and productivity of Cu-contaminated soils over a five-year period. The results underscore the effectiveness of specific treatments, particularly compost (C5) and compost pellets (Pt5) at the 5% addition rate, in enhancing soil quality and crop yield. These treatments stabilized critical soil parameters, including pH, CEC, and P availability, which are essential for sustainable cropping, improved nutrient cycling, and reduced the bioavailability of excess metals. In particular, the use of compost-based treatments contributed to lowering Cu mobility over time, confirming their potential for risk mitigation in moderately contaminated soils. The Pt5 and C5 treatments also enriched the soil with beneficial bacterial genera that were positively correlated with winter barley yield and nutrient-rich soil properties. Overall, the C5, Pt5, and Pt2 treatments significantly increased winter barley yield. The five-year benefits of C5 and Pt5 highlight the enduring positive impact these amendments can have on soil health

and crop productivity. These findings demonstrate that organic amendments, when appropriately selected, can enhance soil fertility, foster sustainable farming practices, and mitigate the effects of soil contamination, thereby promoting crop sustainability even under environmental stress.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-025-00810-1.

Additional file 1.

Author contributions

Conceptualization, CC, GZ, PS, LG, NO, GR, and MM; Formal analysis, CC, GZ and PC; Funding acquisition, PS, and MM; Methodology, CC, GZ, PS, LG, NO, GR, and MM; Writing—original draft, CC and GZ; Writing—review and editing PS, GR and MM; providing amendments, AS, TP, WS, and BR. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials

No datasets were generated or analysed during the current study.

Declarations

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The authors declare no competing interests.

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