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Crop diversification shapes microbial communities and alters carbon-use efficiency across European agricultural soils

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Abstract. Crop diversification practices are expected to influence soil carbon (C) dynamics by altering microbial communities, yet their effects across environmental contexts and on carbon use efficiency (CUE) remain unclear. We tested whether diversified cropping systems modify microbial alpha diversity and community composition, and whether they alter diversity-function relationships. To do so, we analysed fungal and bacterial communities and CUE in soils from six countries along a pan-European gradient. Microbial composition was strongly structured by environmental context, particularly for fungi. After accounting for this context, crop diversification explained a small but significant fraction of variation in both bacterial and fungal communities. However, responses differed between microbial groups, with bacterial communities showing consistent but minor shifts, whereas fungal responses were highly context dependent. Crop diversification did not affect bacterial alpha diversity, measured as richness and Shannon diversity, but had a marginal, context-dependent effect on fungal richness. Although crop diversification did not directly affect CUE, it modified the relationship between fungal Shannon diversity and CUE, with a weak negative association under control conditions which disappeared under crop diversification. This interaction was driven by microbial respiration rather than microbial growth, indicating shifts in C loss

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pathways. Overall, our findings show that crop diversification subtly reshapes microbial communities within strong environmental constraints and can alter diversity-function relationships, highlighting the context dependency of soil C stabilization.

1. Introduction

Soil organic carbon (SOC) represents the largest terrestrial carbon (C) pool and plays a central role in climate change mitigation. Soil microorganisms are key regulators of SOC dynamics through their control of C transformation processes, particularly carbon use efficiency (CUE), which reflects the proportion of assimilated carbon allocated to biomass rather than lost as CO_2 through respiration (Liang et al., 2019). Higher microbial CUE can reduce C losses while enhancing the formation of microbial biomass and by-products that contribute to soil organic matter stabilization via necromass formation.

Microbial community composition and diversity have been identified as important drivers of microbial CUE (Bölscher *et al.*, 2016; Saifuddin *et al.*, 2019; Domeignoz-Horta *et al.*, 2020). More diverse communities may enhance resource use efficiency through a broader range of metabolic capabilities and functional complementarity. However, the relationship between diversity and CUE is not universal and appears highly context-dependent, varying with environmental conditions such as nutrient availability, temperature, and moisture, as well as biotic interactions including competition and metabolic trade-offs (He *et al.*, 2024).

At the same time, agricultural intensification and cropland expansion have contributed to significant SOC losses. In response, policies such as the EU Common Agricultural Policy promote sustainable practices, including crop diversification. Increasing plant diversity has been shown to influence soil microbial communities by enhancing resource heterogeneity and altering plant-soil interactions (Domeignoz-Horta *et al.*, 2024; Zhou *et al.*, 2024). Soil microbial communities in agricultural ecosystems are shaped by abiotic conditions such as pH, soil structure and moisture (Lauber *et al.*, 2013; Philippot *et al.*, 2013), biotic factors such as crop species (Ladygina & Hedlund, 2010) , and agricultural practices (Hartman *et al.*, 2018; Babin *et al.*, 2019; Jaeger *et al.*, 2023). Moreover, agriculture can induce significant taxonomic and functional shifts in both bacteria (Peng et al., 2024) and fungi (Edlinger *et al.*, 2022).

While crop diversification is often associated with increased microbial diversity (Alahmad *et al.*, 2019; Kumar *et al.*, 2024), its effects on microbial functioning, particularly CUE, remain unclear and may depend on management intensity and environmental context. For example, intercropping has been shown to enhance microbial interactions and increase CUE (Domeignoz-Horta *et al.*, 2024), whereas less diverse cover crop systems can reduce microbial diversity and abundance (Wang et al., 2020). Despite growing evidence at local scales, it remains unresolved whether crop diversification consistently influences microbial communities and CUE across broader environmental gradients.

The objectives of the presented study were to i) assess how crop diversification influences microbial communities across environmental contexts, by quantifying its effects on community composition and diversity, and evaluating the consistency and context dependency of these responses; and ii) evaluate whether crop diversification modifies biodiversity-ecosystem functioning relationships, specifically the relationship between microbial diversity and CUE, and test the relative contributions of microbial growth and respiration to this relationship.

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2. Methods

2.1. Sample sites & Carbon Use Efficiency quantification

Top soils (0-20 cm) were sampled at six sites along a pan-European climate gradient from Sweden (Aronsson & Torstensson, 1998; Poeplau & Don, 2015), the Netherlands (Elhakeem *et al.*, 2023), France (Hu & Chabbi, 2022), Austria, Slovenia, and Spain. Sites, experimental designs and sampling strategies have been described in detail in (Schroeder *et al.*, 2025). At each site, a control and one diversified treatment were sampled. The CUE data collected from Schroeder *et al.* (2025) was used for the correlations in the presented study.

2.2. Assessment of microbial community composition and diversity

DNA was extracted from the 58 soil samples using the DNeasy PowerSoil Pro kit (Qiagen). Analysis of the diversity and composition of the prokaryotic, and fungal communities were performed by Allgenetic (Spain) using MiSeq (Illumina 2 × 300 bp) sequencing with the following primers: 515F (5'- GTGYCAGCMGCCGCGGTAA-3')(Parada *et al.*, 2016) and 806rR (5'- GGACTACNVGGGTWTCTAA -3') (Apprill *et al.*, 2015) for bacteria, and gITS7 (5'-GTGARTCATCGARTCTTTG-3') (Ihrmark *et al.*, 2012) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990) for fungi. Raw sequences were analysed using QIIME2 (Quantitative Insights Into Microbial Ecology 2) (Bolyen *et al.*, 2019). Chimeras were removed by means of the DADA2 plugin (Callahan *et al.*, 2017) and then sequences were denoised into amplicon sequence variants (ASVs). ASVs taxonomic assignment was achieved using the UNITE database (v25.07.2023)(Abarenkov *et al.*, 2024) for fungi, and the SILVA database (v138.1) (Quast *et al.*, 2013; Yilmaz *et al.*, 2014) for prokaryotes. The raw sequences are available from SRA BioProject ID PRJNA1222789 (Prokaryotes) and PRJNA1222825 (Fungi). The outputs of the QIIME2 pipeline were imported into Rstudio (RStudio Team. R: A Language and Environmental for Statistical Computing, 2021) and used to create a phyloseq object with the R package qiime2R v.0.99.6 (Bisanz, 2018).

All statistical analyses were performed using R version 4.5.3 (R Core Team, 2020) and RStudio version 2026.1.1.403 (Posit Team, 2026). For downstream analyses, bacterial and fungal ASV tables were rarefied to 11,865 and 34,459 sequences per sample, respectively. Richness (number of taxa observed) and Shannon diversity (accounts for both taxon richness and relative abundance/evenness) index were calculated using the phyloseq package (McMurdie & Holmes, 2013). To address objective (i), namely assessing how crop diversification influences microbial communities across environmental contexts, we first quantified its effects on community composition using partial distance-based redundancy analysis (partial dbRDA) on Bray-Curtis dissimilarities of Hellinger-transformed data, conditioning on country-season context, with the vegan package (Oksanen *et al.*, 2015). The consistency of treatment effects was further assessed using a leave-one-context-out (LOCO) sensitivity analysis approach, in which each country-season combination was sequentially excluded and models refitted.

We then assessed responses of microbial alpha diversity to crop diversification using linear mixed-effects models fitted with the lme4 package (Bates *et al.*, 2015). Crop diversification was included as a fixed effect and country-season context as a random effect. Context dependency was evaluated using random-slope models allowing both intercepts and diversification effects to vary among contexts (microbial diversity ~ diversification + (1 + diversification | country-season)). Models were fitted by restricted maximum likelihood, and assumptions of normality and homoscedasticity were assessed using DHARMa (Hartig, 2022). The significance of the fixed effect

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was assessed using Type III χ^2 tests implemented in the car package (Fox & Weisberg, 2019). Context-specific effects were extracted as best linear unbiased predictions (BLUPs) of diversification slopes, with 95% confidence intervals obtained via parametric bootstrapping (lme4::bootMer, 1,000 simulations). Model-based predictions for each treatment and context were derived by combining fixed and random effects, with uncertainty likewise derived from bootstrapping.

To address objective (ii), we evaluated whether crop diversification modifies biodiversity-ecosystem functioning relationships by fitting linear mixed-effects models relating microbial diversity to CUE, with diversity, diversification, and their interaction as fixed effects and country-season as a random intercept (CUE ~ microbial diversity × diversification + (1 | country-season)). Random slopes were not retained because they did not improve model performance and increased model complexity. The significance of fixed effects was assessed using Type III χ^2 tests. Treatment effects and differences in diversity-CUE slopes between diversification treatments were evaluated using estimated marginal means and marginal trends with the emmeans package (Lenth & Piaskowski, 2026). Finally, to determine whether observed CUE patterns were driven primarily by microbial growth or respiration, the same modelling framework was applied separately to C growth and C respiration. The consistency of significant interaction effects was further assessed using LOCO sensitivity analysis as above. Results were visualized using model-predicted marginal effects.

All figures were produced using the tidyverse metapackage (Wickham *et al.*, 2019) and multi-panel figures were assembled using cowplot (Wilke, 2025).

3. Results & Discussion

3.1 Microbial community responses to crop diversification across environmental contexts

Partial db-RDA revealed contrasting patterns between bacterial and fungal communities (Fig.1). Crop diversification explained a similarly small proportion of total community variation in both groups, accounting for 1.6% ($p = 0.007$) in bacteria and 1.7% in fungi ($p = 0.001$) after controlling for country-season effects. However, the amount of variation attributed to country-season differed markedly between microbial groups (Fig.1A).

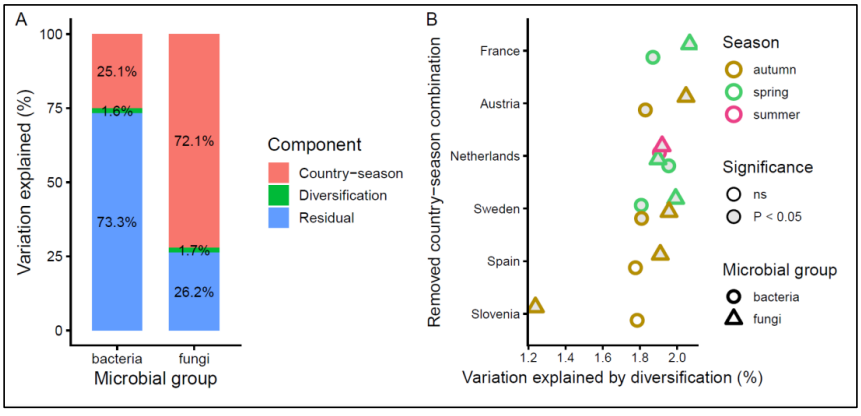

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Figure 1. Relationship between crop diversification and soil microbial community composition. (A) Variance partitioning showing the proportion of variation in bacterial and fungal community composition explained by country-season combinations, crop diversification, and residual variation. Country-season effects accounted for most of the variation, whereas crop diversification explained a small but detectable fraction of compositional variation in both microbial groups. (B) Leave-one-country-season-out sensitivity analysis showing the percentage of variation in microbial community composition explained by crop diversification after sequentially removing each country-season combination.

Country-season explained 25.1% of bacterial community variation but 72.1% of fungal community variation, indicating that fungal communities were much more strongly structured by broad spatial-temporal context. In contrast, residual variation was much higher for bacteria, suggesting greater within-context heterogeneity or stronger influence of unmeasured local soil and management factors. Leave-one-context-out analyses further indicated that the fungal diversification effect was robust to the removal of individual country-season combinations (Fig.1B), whereas the bacterial effect became non-significant when Spain autumn or Slovenia autumn were excluded. Thus, crop diversification was associated with modest compositional shifts in both microbial groups, but this signal was more stable in fungi than in bacteria. These contrasting patterns may reflect fundamental ecological differences between the two microbial groups. Bacterial communities are often strongly structured by local edaphic conditions, particularly soil pH, which may contribute to the high residual variation observed after accounting for country-season effects (e.g. Fierer & Jackson, 2006). By contrast, fungal communities often show pronounced biogeographic and environmental structuring, and many fungal taxa are closely linked to plant-derived resources and host associations (e.g. Tedersoo *et al.*, 2014). This may explain the much larger country-season component observed for fungi.

Crop diversification had no effect on bacterial alpha diversity and only a marginal effect on fungal richness (LMM, $\chi^2 = 3.28$, $p = 0.07$; Fig.2A). Diversification tended to increase fungal richness, but the effects were moderate in most contexts (+5-40 taxa) but markedly stronger in Slovenia (+ ca. 129 taxa; Fig.2B). The strong Slovenian response likely reflects differences in management practices. At this site, permanent natural vegetation was maintained between crop rows in diversified treatments, whereas control plots were tilled and kept bare. Previous work has shown that tillage can increase bacterial diversity but reduce fungal diversity in vineyard systems (Pingel *et al.*, 2023), highlighting the sensitivity of fungi to disturbance. Thus, the increase in fungal richness observed in Slovenia is likely driven by reduced soil disturbance under crop diversification.

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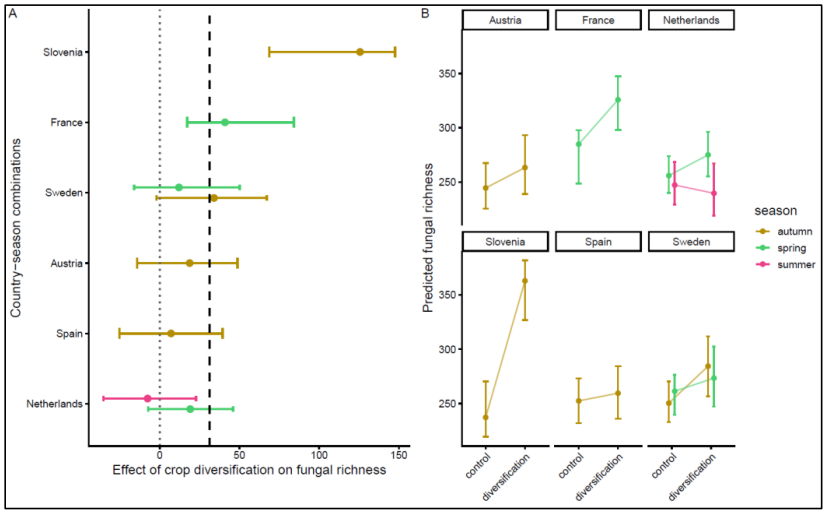

Figure 2. Context-dependent effects of crop diversification on fungal richness. (A) Country-season-specific effects of crop diversification on fungal richness. Points represent the predicted diversification effect for each country-season combination using a linear mixed-effects model with random slopes, and error bars show bootstrapped 95% confidence intervals. The dashed vertical line indicates the overall fixed effect of crop diversification on fungal richness across all country-season combinations, while the dotted vertical line marks zero, corresponding to no differences in richness between diversified and control soils. Confidence intervals that do not overlap the zero line indicate statistically significant differences. (B) Model-based predictions of fungal richness under control and diversification treatments for each country-season combination. Lines connect treatment levels within each country-season to highlight the magnitude and direction of diversification effects.

Positive relationships between crop diversity and microbial diversity have been widely reported (e.g., (Alahmad *et al.*, 2019; Kumar *et al.*, 2024), often attributed to increased heterogeneity in root exudates and root architectures under crop diversification (Vukicevich *et al.*, 2016). The contrast between fungal and bacterial responses to crop diversity observed here are also consistent with previous studies (Pingel *et al.*, 2023; Tang *et al.*, 2024) and likely reflect, once again, fundamental ecological differences. Bacterial diversity is largely governed by soil physicochemical conditions and tends to be relatively resilient to disturbance (e.g., Bahram *et al.*, 2018), whereas fungal diversity is more tightly linked to plant-derived resources and is more sensitive to agricultural disturbance such as tillage (e.g., Banerjee *et al.*, 2019). Accordingly, diversification, particularly when associated with reduced disturbance, can enhance fungal richness. The lack of change in Shannon diversity further suggests that this effect is driven by the addition of low-abundance taxa rather than shifts in community dominance, which is supported by the cumulative rank-abundance curves of fungal communities (Fig.S1). This aligns with recent findings that agricultural intensification reduces fungal diversity and rare taxa (Banerjee *et al.*, 2024).

3.2. Crop diversification modifies diversity-carbon use efficiency relationships

CUE represents a central control point in soil C cycling, as it determines the proportion of assimilated C allocated to growth versus respiration. Higher CUE promotes microbial biomass production and residue formation, thereby

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enhancing long-term C stabilization; whereas lower CUE leads to greater respiratory losses and reduced C stabilization potential.

Crop diversification did not directly affect CUE but instead modified the relationship between fungal diversity and ecosystem functioning. Specifically, it modified the relationship between fungal Shannon diversity and CUE (LMM, $\chi^2 = 4.80$, $p = 0.028$; Fig.3A), with a steeper slope under control than diversification ($p = 0.035$). Because Shannon diversity reflects both richness and evenness, these results suggest that CUE may be more strongly related to shifts in the relative abundance of dominant fungal taxa than to richness alone. While diversification increased fungal richness, the additional taxa may have been relatively rare and therefore contributed little to overall carbon processing. Consequently, variation in carbon-use efficiency may be more closely associated with changes in the dominance structure of fungal communities. The LOCO sensitivity analysis further showed that the interaction effect was generally robust across country-season combinations, with positive differences in slopes observed in all cases, although the magnitude of the effect varied among contexts and statistical significance was not consistently retained (Fig.3B). This indicates that the observed modification of the diversity-function relationship was not driven by a single country-season combination but reflects a broader pattern across the study system. No relationship was detected between bacterial diversity and CUE, nor did crop diversification modify bacterial diversity-function relationships, consistent with the weaker response of bacterial diversity to diversification and its stronger regulation by environmental conditions.

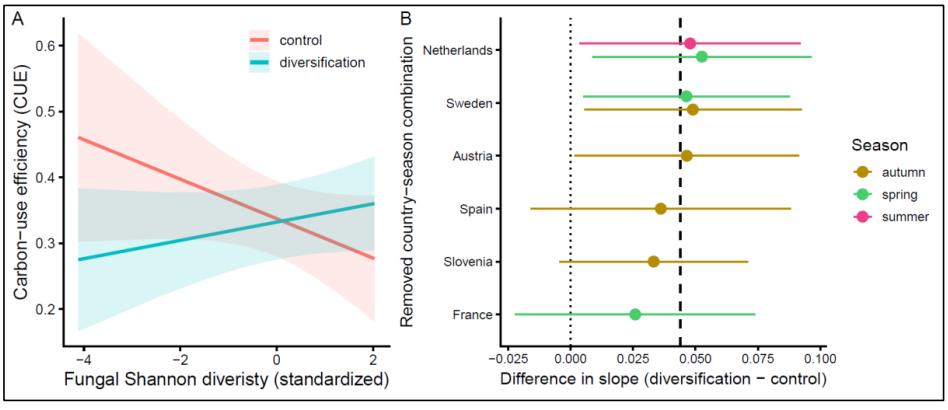

Figure 3. Drivers of microbial carbon-use efficiency (CUE). (A) Relationship between standardized fungal Shannon diversity (mean=0, SD=1) and CUE under control and crop diversification treatments. Lines represent model-predicted marginal effects from linear mixed-effects models including fungal Shannon diversity, crop diversification, and their interaction as fixed effects, with country-season as a random intercept. Shaded areas indicate 95% confidence intervals. CUE showed a significant interaction between diversity and diversification (LMM, $\chi^2 = 4.80$, $p = 0.028$), indicating that the effect of diversification on CUE depended on diversity levels. (B) Leave-one-country-season-out sensitivity analysis of the interaction between fungal Shannon diversity and crop diversification on CUE. Points show the estimated difference in Shannon-CUE slopes between diversified and control soils after removing each country-season combination, and error bars indicate 95% confidence intervals. The dashed vertical line represents the full-model estimate, while the dotted vertical line marks zero, corresponding to no difference in slopes between diversified and control soils. Confidence intervals that do not overlap the zero line indicate statistically significant differences.

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Mechanistically, the treatment-dependent relationship between fungal Shannon diversity and CUE was not primarily explained by changes in microbial growth. Although C growth increased under diversification (LMM, $\chi^2 = 7.03$, $p = 0.008$), it was not significantly related to fungal diversity ($p = 0.089$), and the fungal diversity × diversification interaction was not significant ($\chi^2 = 0.42$, $p = 0.51$; Fig.4A). In contrast, C respiration showed a strong fungal diversity × diversification interaction ($\chi^2 = 12.19$, $p < 0.001$; Fig.4B), with slopes differing significantly between treatments ($p = 0.001$). This pattern mirrored, but was opposite in direction to, the relationship observed for CUE, indicating that variation in CUE was mainly driven by changes in respiratory C losses rather than by changes in microbial C assimilation. The LOCO sensitivity analysis supported this interpretation, as the difference in respiration slopes between diversification and control remained consistently negative after sequentially removing each country-season combination, although the magnitude of the effect varied among contexts (Fig.S2). Together, these results suggest that crop diversification modifies how fungal communities are associated with C loss pathways, rather than altering the relationship between fungal diversity and microbial growth.

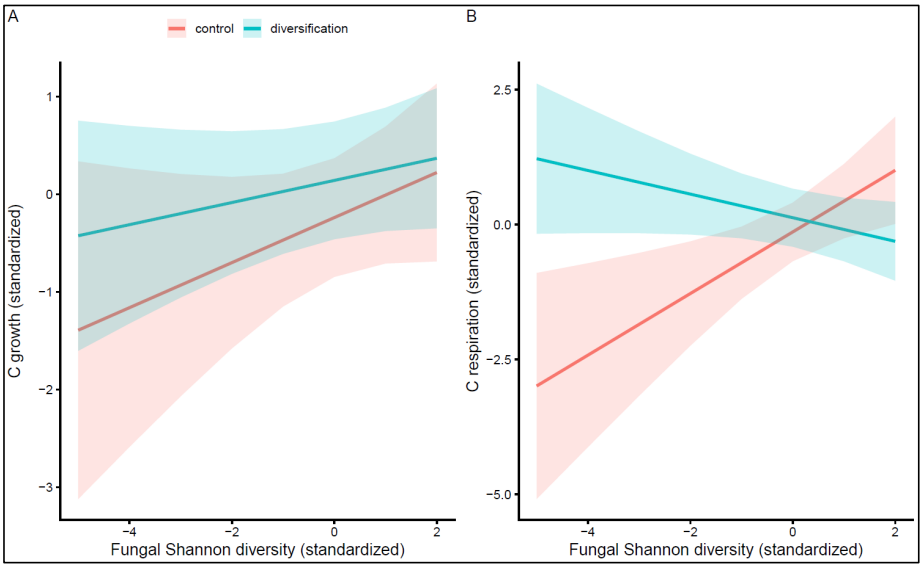

Figure 4. Effects of fungal Shannon diversity and crop diversification on (A) microbial growth (C growth) and (B) microbial respiration (C respiration). Lines represent model-predicted marginal effects from linear mixed-effects models including fungal Shannon diversity, crop diversification, and their interaction as fixed effects, with country-season as a random intercept. Shaded areas indicate 95% confidence intervals. For C growth, crop diversification increased overall values but did not modify the diversity relationship. In contrast, C respiration showed a significant interaction between diversity and diversification (LMM $\chi^2 = 12.19$, $p < 0.001$), indicating that respiration drives the interaction previously observed for CUE (Fig.3).

Beyond the effects of crop diversification, CUE showed strong country-season-level structuring (Fig.S3), indicating substantial variability among countries and across seasons within countries. For example, CUE was notably higher in the Netherlands during spring compared to summer and higher in Sweden during spring

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compared to autumn. These patterns highlight the importance of both spatial and temporal dynamics in regulating microbial C allocation, likely reflecting seasonal shifts in soil moisture, temperature, and substrate availability. Consequently, the potential for C stabilization appears highly context-dependent, with periods of elevated CUE favouring microbial biomass production and residue formation.

These results suggest that the effects of crop diversification on C stabilization are primarily indirect. Although diversification consistently altered bacterial and fungal community composition, these shifts were small relative to the strong influence of environmental filtering, suggesting that diversification does not fundamentally reorganize communities but instead subtly redistributes taxa within existing constraints. If such shifts favour taxa with higher biomass production efficiency (e.g., slower-growing, resource-acquisitive organisms), even modest compositional changes could influence long-term C stabilization without producing large immediate changes in bulk CUE. Recent evidence that communities with a higher proportion of larger-bodied eukaryotic microorganisms exhibit higher CUE (Dang & Morrissey, 2024) further supports the idea that subtle compositional shifts can translate into altered C-use strategies.

Altogether, our findings align with broader ecosystem patterns while highlighting strong context dependency in diversity-function relationships. In temperate forest soils, prokaryotic richness is positively associated with CUE, whereas fungal richness shows no clear relationship (Domeignoz-Horta *et al.*, 2020). In contrast, our results show that fungal diversity influences CUE only under specific management conditions, indicating that these relationships depend on environmental and disturbance regimes. Forest soils are relatively undisturbed and nutrient-limited, favouring oligotrophic, slow-growing taxa and consistently high efficiency. By comparison, arable soils experience frequent disturbance and pulses of labile substrates, promoting copiotrophic taxa with higher metabolic rates and lower baseline CUE, thereby increasing the potential for management to modulate diversity-function linkages.

To further elucidate the mechanisms that govern these patterns, future work should integrate functional trait approaches and targeted experiments using synthetic microbial communities. In addition, establishing land-use-specific microbial baselines through long-term diversification experiments (e.g., cover crops and rotations) will be essential for improving the transferability of CUE-diversity relationships across contrasting ecosystems.

4. Conclusions

Our results show that crop diversification does not directly increase microbial carbon-use efficiency (CUE) but instead modifies fungal diversity-function relationships. Microbial community composition was mainly structured by country-season conditions, while diversification explained only a small fraction of variation. However, diversification produced detectable compositional shifts and, in some contexts, clear increases in fungal richness, particularly where management disturbance was reduced.

These changes did not translate into higher CUE overall. Rather, diversification weakened the negative relationship between fungal Shannon diversity and CUE observed under control conditions, suggesting that it alters how fungal communities mediate soil carbon processing. This pattern appeared to be driven primarily by changes in respiration rather than microbial growth, indicating that diversification influences respiratory carbon losses more than carbon assimilation.

Overall, crop diversification may not universally enhance microbial CUE, but it can reorganize fungal diversity-function relationships. Even small and context-dependent shifts in fungal communities may therefore have

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functional consequences for soil carbon cycling and help stabilize carbon processing across environmental contexts.

5. Author contributions

AV, EL, SV, AM and BAJ conceived the study. All authors contributed to the study design and methodology. JS and AV carried out the experimental work. AV and SV performed the statistical analyses. AM led funding acquisition. AV and BAJ wrote the original draft, and all authors reviewed, edited and approved the final manuscript.

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8. Conflict of interest

The authors declare there are no competing interests to declare.

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