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Impact of perennial forage plants on microbial carbon use efficiency in weathered Ethiopian soils

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Abstract

Aims: Ethiopian soils are highly weathered and in urgent need of remediation. Increasing the soil carbon (C) pool by perennials potentially remediates soil degradation while sequestering atmospheric CO_2. The soil microbial loop is thought to play an important role in soil C sequestration as it contributes significantly to the stabilization of plant litter C. The amount of stabilized litter C has been hypothesized to depend on microbial carbon use efficiency (mCUE), which is sensitive to soil nutrients and plant inputs. Here, we investigate the effect of two perennial forage crops on mCUE in four weathered Ethiopian soils.

Methods: We cultivated the grass *Urochloa* cv. Cayman as single stand and in mixture with the legume *Desmodium intortum* in a fully factorial greenhouse experiment with four distinct farmers' soils from the Ethiopian Rift Valley (Amhara and Sidama). Microbial CUE was assessed after 104 days of plant growth using ^{18}O-H_2O incorporation.

Results: Microbial CUE varied between the four soils, with Amhara soils showing lower mCUE than Sidama soils. Overall, mCUE was little affected by plant growth but increased significantly with plant dry weight (DW) in one of the two Sidama soils. Mass specific growth rates remained small in Amhara soils, indicating that factors other than plant C input inhibited microbial growth. Plant composition had no effect in Amhara soils while the *Urochloa* × *Desmodium* mixture tended to have lower mCUE in Sidama soils.

Conclusions: Perennials may improve mCUE in weathered soils, but this stimulation strongly depends on soil nutrient availability and suggests that there are critical nitrogen (N) and phosphorous (P) thresholds below under which mCUE does not respond to growing plants.

This implies that highly degraded soils must be ameliorated before using perennials to stimulate nutrient cycling and restore soil health.

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1. Introduction

Soils store vast amounts of carbon (C) and can sequester additional C from the atmosphere (Dynarski et al., 2020). In sub-Saharan Africa, many soils are severely weathered and limited in nutrients due to intensive farming practices, overgrazing and little return of crop residues, highlighting the need to develop ameliorative land management practices (FAO, 2019; Gebreselassie et al., 2016). Plant based approaches, particularly the targeted use of perennial mixtures in crop rotations, have been demonstrated to provide multiple benefits by reducing soil erosion and building soil organic carbon (SOC) over time (Fornara and Tilman, 2008; Fry et al., 2026). However, the stabilization and fate of plant C in soils largely depend on the activity and growth dynamics of soil microbial communities (Malik et al., 2019; Ziegler and Billings, 2011), which also play a key role in soil nutrient cycling.

Plant C entering the soil is processed through a microbial loop of growing and decaying microbial cells allocating decomposed C to either microbial biomass, respired CO_2 or exoenzyme synthesis. The proportion of C that is assimilated into microbial biomass relative to the amount lost by respiration is defined as the apparent microbial carbon use efficiency (mCUE) (Sinsabaugh et al., 2013). Higher mCUE values reflect relatively more allocation of C to microbial biomass, which can be interpreted as microbial growth (Spohn et al., 2016). Upon cell death, necromass is formed which is prone to stabilization on mineral and metal oxide surfaces, thus increasing the soil SOC content (Zhou et al., 2023). Strategies aimed at increasing mCUE can therefore increase sequestration of atmospheric CO_2 as soil organic carbon (SOC).

Microbial CUE has been shown to be positively influenced by microbial growth (Zheng et al., 2019). Soil properties that inhibit microbial growth, such as limited nitrogen (N) and phosphorous (P) availability, elevated aluminum (Al), low pH and fine soil texture can therefore reduce CUE (Cotrufo et al., 2013; Jones et al., 2019). Also, microbial community composition affects CUE, as copiotrophic microorganisms tend to have a lower CUE than oligotrophic ones (Malik et al., 2019). Under optimal conditions, the theoretical maximum mCUE is 0.88, accommodating for an inherent respiratory loss of ~12 % of C taken up (Gommers et al., 1989). However, such values are rarely achieved in natural systems. In multi-resource limited environments, mCUE values are closer to 0.3, as microbial communities prioritize maintenance metabolism over growth. In these scenarios, a substantial proportion of microbial C and N has to be allocated to nutrient acquisition, which is

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energetically costly (Sinsabaugh et al., 2013). Empirical studies have demonstrated that N and P additions increase mCUE by promoting microbial C incorporation without a corresponding increase in respiration (Poeplau et al., 2019; Spohn et al., 2016). Increased nutrient availability generally improves mCUE by reducing microbial investment in exoenzyme synthesis and redirecting resources to microbial growth (Manzoni et al., 2012; Sinsabaugh et al., 2013).

In extensive crop production, plant mediated approaches instead of fertilizer inputs may provide an alternative way to ameliorate soils. For instance, increasing plant species diversity has been shown to increase soil C and N in the long run (Fornara and Tilman, 2008), with SOC content being positively correlated with microbial biomass C (C_{mic}) (Prommer et al., 2020). In terms of mCUE, the C:N ratio of plant residues may play a critical role; low C:N residues such as from legumes are associated with high mCUE, apparently because they relieve co-limitation of N for microbial growth. More generally, increasing plant C and N input into soil by intercropping perennials may lead to priming, i.e. increased microbial decomposition of native SOC, which promotes microbial growth and mCUE, thereby balancing the loss of SOC (Kuzyakov, 2010; Manzoni et al., 2012). Likewise, co-cultivation of plants with complementary growth traits in mixtures may have positive side effects on microbial growth and mCUE as mixtures may overyield, develop more roots and stimulate C and N inputs and cycling (Fornara and Tilman, 2008; Zhang et al., 2021). The suitability of mCUE as a sensitive indicator for monitoring belowground effects of plants on microbial nutrient status, functioning and soil health in degraded soils remains to be tested.

We conducted a fully factorial greenhouse experiment to test the effects of two forage species on mCUE in four weathered Ethiopian soils. We grew the native grass *Urochloa* hybrid cv. Cayman in single stands and in mixture with the non-native legume *Desmodium intortum* in four soils collected from four farmers' training centers (FTCs) situated in two pedoclimatically contrasting regions of the Ethiopian Rift Valley (Amhara and Sidama region). We hypothesized that (i) mCUE and its response to plant inputs would strongly depend on initial soil properties with limited response in highly nutrient poor soils, (ii) legume inclusion would increase mCUE by relieving N co-limitation and (iii) plant functional diversity would exert a greater influence on mCUE than overall plant biomass.

2. Materials and Methods

2.1 Experimental sites and soils

Soils were collected from four Farmers' Training Centers (FTCs) in Ethiopia, which are managed jointly by local farmers and extension services. All soils were highly weathered acidic and nutrient poor (Table 1). In Sidama (Southern Ethiopia), soils were collected at Boricha (1837 m.a.s.l.) and Bilate (1819 m.a.s.l.), and in Amhara (Northern Rift Valley) at Kudmi (1983 m.a.s.l.) and Abiyot Fana (2043 m.a.s.l.). Mean annual rainfall and temperature in the Sidama region are 650 to 960 mm and 22 °C to 30 °C, respectively, and in the Amhara region 1500 to 2200 mm and 24 °C to 27 °C. All fields were under maize cultivation with a fertilizer application of 64 kg urea N ha^{-1} and 20 kg ha^{-1} P as di-ammonium phosphate (DAP). Soils for the greenhouse experiment were sampled in June 2021 at all four sites from a depth of 0-25 cm. Five composite soil samples, consisting of 5-8 subsamples each, were taken per field site, one from each quarter and one from the center of the field. Larger roots and stones were removed, and the soil was air-dried before sending it to the Norwegian University of Life Science for the greenhouse experiment.

2.2 Greenhouse experiment

The soil samples were air dried and shipped to Norway, where pooled soils from the same site and sieved (2 mm). We used slightly conical 1 L pots with a height of 11 cm and a rim diameter of 13 cm. A filter cloth and a 1 cm sterile sand layer were placed in the bottom to allow for free drainage of excess water. The pots were filled with ~1 L of dry soil which was rewetted to field capacity and left to preincubate for 48 h before we sowed the seeds directly into the pots. We used two plant species in the experiment; the native grass *Urochloa* and the non-native legume *D. intortum* in two different treatments: either as *Urochloa* single stands or *Urochloa* × *Desmodium* mixture. The *Urochloa* seeds were obtained from Tropical seeds, LLC (Papalotla group, Mexico) and the *D. intortum* seeds from the Alliance Biodiversity & CIAT (Kenya). With four soils, two plant treatments and five replicates, a total of 40 pots were set up. Ten seeds were sown per pot in a pentagram shape to ensure good spatial distribution. For the grass-legume mixture, five seeds of each species were sown evenly distributed across the pot surface. Four extra pots with only soil (one per site) were prepared as unplanted controls. The pots were placed in a greenhouse with a day/night light cycle of 16/8h and a temperature cycle of 25/15°C. The placement of the pots was reshuffled every 4.5 weeks. The pots were watered daily with 40 mL tap water using an automatic drip

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Table 1. Chemical and physical characteristics of the four soils used in the greenhouse experiment. pH, organic C, total N and C:N ratio are given as average and standard deviation (SD) based on a sample size of n = 5

			%			mg kg^{-1}		cmol kg^{-1}		%		
	Site	pH	C_{org}	N_{tot}	C:N ratio	P	Al	CEC	Sand	Silt	Clay	Soil type
Amhara	Kudmi	5.53 ± 0.17	2.64 ± 0.15	0.243 ± 0.017	10.9 ± 0.32	1.30	930	33.4	7.00	46.7	46.3	Silt Clay
	Abiyot Fana	5.26 ± 0.092	2.10 ± 0.11	0.188 ± 0.015	11.2 ± 0.38	1.00	1200	36.4	10.7	66.3	23.0	Silt Loam
Sidama	Boricha	6.26 ± 0.10	2.17 ± 0.22	0.183 ± 0.024	11.9 ± 0.36	15.0	640	48.5	36.6	54.4	9.00	Silt Loam
	Bilate	6.20 ± 0.041	1.82 ± 0.11	0.155 ± 0.011	11.8 ± 0.29	16.0	580	37.3	41.7	49.0	9.30	Loam

watering system regulated by a self-built PLS system controlling a water pump (SIP SUB 1075-FS, SIP industrial, England).

2.3 Sample collection

The plants were cut 104 days after sowing 1 cm above the soil and dried at 60°C for 48 hours before weighing them. Soil samples were taken in the center of the pot down to the sand layer with a spoon. The soil was sieved with a 2 mm mesh size and stored at 4°C in the dark before further analysis.

2.4 Laboratory analysis

We determined soil water content (WC) by weight difference after drying the soils at 60°C for 48 h. All samples were adjusted to 30% gravimetric soil moisture before analyzing them.

2.4.1 Respiration

We measured soil respiration by weighing soil equivalent to 1 g dry weight (DW) into 10 mL vials (Chromacol) closed with crimp-sealed butyl rubber septa. The vials were flushed five times with synthetic air (21vol% O_2 and 79 vol% N_2). After releasing over-pressure, the vials were left to incubate for 24 h at room temperature (22 °C) before measuring CO_2 headspace concentration by a gas-chromatograph (GC model 7890A, Agilent, USA). CO_2 production was calculated as CO_2-C g^{-1} h^{-1}.

2.4.2 Microbial biomass C

We assessed microbial biomass C (C_{mic}) by chloroform fumigation-extraction (Brookes et al., 1982). Four grams of soil adjusted to 30% gravimetric soil moisture were weighed into aluminum cups and placed in a desiccator together with 25 mL of chloroform in a beaker. The desiccator was evacuated three times to enrich the atmosphere with chloroform and left in the dark to incubate for 24 h. After pumping away excess chloroform, the soils were transferred to Falcon tubes and suspended in 20 mL of 0.5 mol L^{-1} K_2SO_4. The tubes were shaken horizontally for 1 h. Parallel to the fumigation, non-fumigated samples were prepared following the same procedure. The samples were filtered through Schleicher & Schuell paper filters, and the concentration of dissolved organic carbon (DOC) was measured on a TOC analyzer (TOC Vcph/ CPN, Shimadzu, Japan). C_{mic} was calculated as the difference of DOC concentration between the fumigated and non-fumigated sample. A conversion factor of 0.45 was used (Joergensen, 1996).

2.4.3 ^{18}O-labelling and DNA extractions

To estimate microbial carbon use efficiency (mCUE), we used the ^{18}O labeling method as devised by Spohn et al., (2016). In short, we weighed 300 mg DW equivalent of each soil into 1 mL Cryotubes (Nunc A/S, Denmark). Two replicates of each sample were prepared, one to be labelled with ^{18}O and one to remain non-labeled. Based on the targeted gravimetric water content of 30%, we added different amounts of a 97 at% ^{18}O stock solution to reach 20 at% ^{18}O enrichment in the soil solution. The non-labeled control soil received an equivalent amount of DI-H_2O. The vials were sealed with a screw cap and incubated at room temperature (22 °C). After 24 h, the samples were snap frozen in liquid N_2 and stored in a -80 °C freezer until DNA-extraction.

To maximize the amount of extracted DNA we added 40 mg of skimmed milk powder to restrict binding of DNA to clay particles (Yankson and Steck, 2009). DNA was extracted from both labelled and non-labelled samples using the DNeasy PowerSoil Pro Kit (QIAGEN, Germany) following the standard protocol with the following modifications: the cell lysing was extended to 2 × 40 seconds on a tissue lyser with a 5-minute rest on ice between the cycles and the subsequent centrifugation step was extended to 15 min. Elution was done with 2 × 50 µL elution buffer. DNA concentration was quantified using a Qubit dsDNA high sensitivity assay (Invitrogen, USA). 60 µL of the eluate was transferred to silver capsules and dried at 60 °C for 12 h. To keep the DNA dry until ^{18}O analysis, the capsules were stored in a desiccator.

The ^{18}O isotope ratios of labelled and non-labelled DNA extracts were analyzed at Norway's National Light Stable Isotope Facility, FARLAB, at the University of Bergen (UiB) by pyrolyzing the DNA extract using a Thermo Fisher Scientific Flash 2000-HT PLUS element analyzer (USA) coupled to an isotope ratio mass spectrometer (IRMS, Delta V PLUS, Thermo Fisher scientific, USA). Certified standards of benzoic acid and barium sulfate were used for scale correction. All data were drift and size corrected.

2.5 Calculations

Following Schroeder et al., (2021), newly produced DNA was calculated as

$$\mathrm{DNA_{produced}} = \mathrm{DNA\ O} \times \frac{\mathrm{DNA^{18}O}}{\mathrm{E\%}} \times \frac{100}{31.21} \qquad (1)$$

where DNA O is the amount of O in the DNA elution buffer, DNA^{18}O the at% ^{18}O excess of the labelled sample, E% the ^{18}O enrichment in the soil solution and 31.21 the average weight % of O in DNA ($C_{39}H_{44}O_{24}N_{14}P_4$). Microbial growth was calculated from $\mathrm{DNA_{produced}}$ and measured C_{mic} as

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$$C_{\mathrm{growth}} = \frac{\mathrm{DNA}_{\mathrm{produced}} \times \mathrm{fDNA}}{\mathrm{g\ soil} \times \mathrm{time}} \quad (2)$$

where fDNA is a conversion factor calculated from each sample as C_{mic} (µg C g^{-1} DW soil) per total DNA (µg g^{-1} DW soil) derived from fumigation extraction and Qubit measurements, respectively. Finally, microbial carbon use efficiency was calculated as

$$\mathrm{mCUE} = \frac{C_{\mathrm{growth}}}{C_{\mathrm{growth}} + C_{\mathrm{respiration}}} \quad (3)$$

We also calculated the mass specific growth rate as described by Schroeder et al., (2021) by normalizing $\mathrm{DNA}_{\mathrm{produced}}$ by total DNA:

$$\text{Mass specific growth rate} = \frac{\mathrm{DNA}_{\mathrm{produced}} \times \frac{24}{t}}{\text{total DNA}} \quad (4)$$

2.6 Statistics

We tested the effect of plant treatments, soils and their interactions on mCUE and mass specific growth by two-way ANOVA separately for each response variable (C_{mic}, respiration, mCUE, mass specific growth rate). Statistical differences between treatments were assessed by TukeyHSD. For the effect of plant biomass on C_{mic}, respiration, mCUE and mass specific growth rate, we performed linear regressions. All statistics were done in Rstudio v 4.5.1 (Posit team, 2025).

3. Results

3.1 Plant growth

Aboveground dry weight (DW) biomass differed significantly between the four soils but was more similar between soils from the same region ($p = <0.001$) (Fig. 1). In general, soils from Amhara produced less biomass than soils from Sidama. Biomass was lowest in soil from Abiyot Fana in Amhara and largest in soil from Bilate in Sidama. There were no significant differences in plant dry weight between single stands and mixtures, except for Bilate soil, which supported more growth of *Urochloa* in single stand than in mixture with *Desmodium* ($p = 0.010$). The *Urochloa* single stand in Bilate soil had the highest observed biomass among all plant treatments.

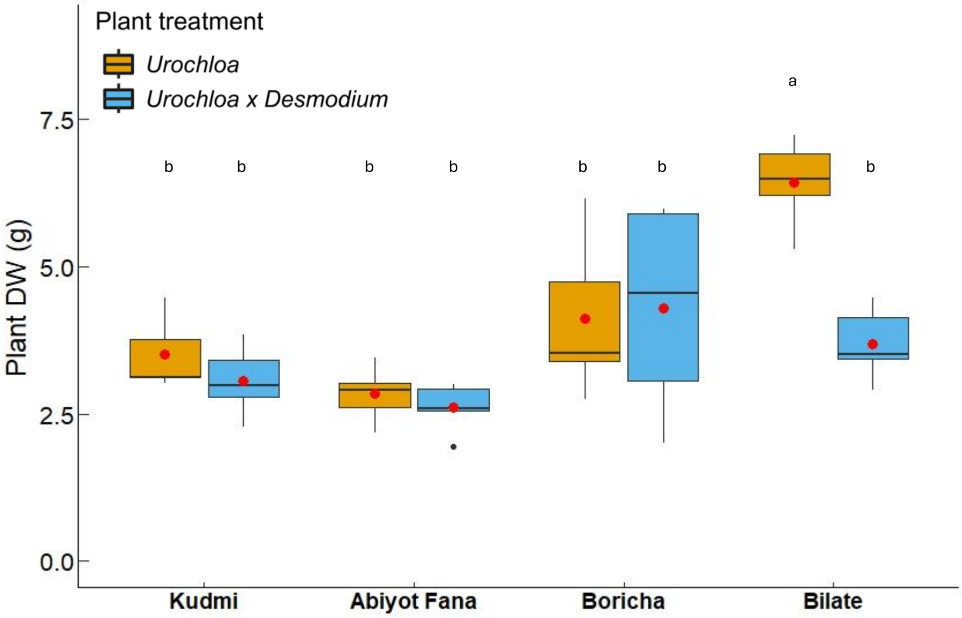

Figure 1. Total dry weight (DW) aboveground biomass per of *Urochloa* single stands and *Urochloa* × *Desmodium* mixtures after 104 d of growth in soils from Kudmi and Abiot Fana (Amhara) and Boricha and Bilate (Sidama). The boxes show upper and lower quartiles with horizontal lines indicating the median. The vertical lines represent the minimum and maximum values and the dots outliers. The red dots represent the arithmetic mean. Different letters indicate statistically significant differences between the treatments and soils. Each box is calculated with a sample size of n = 5.

3.2 Microbial C and soil respiration

C_{mic} varied significantly between the soils ($p = <0.001$) (Fig. 2A) with larger values in Sidama soils, specifically Boricha and smaller values in Amhara soils, especially Abiyot

Fana. On a single pot basis, C_{mic} correlated positively with plant DW in Boricha (p = 0.015) and Bilate (p = 0.016) soils (Fig. 3A). However, there was no significant effect of plant treatments on C_{mic} in any of the soils.

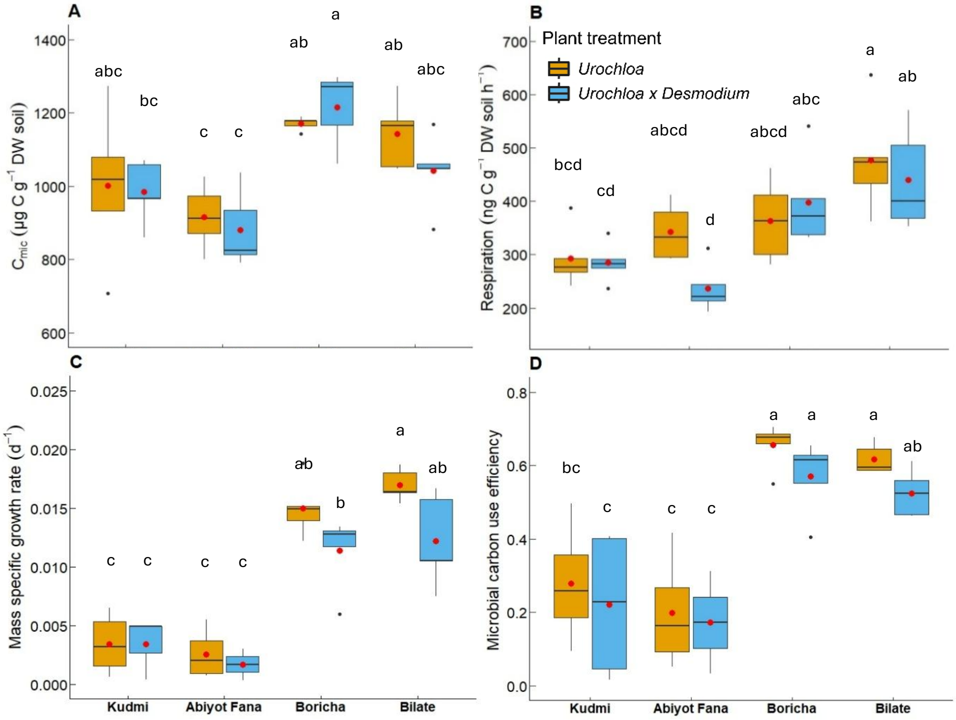

Figure 2. Effects of plant treatments on soil microbial variables: (A) microbial biomass C (C_{mic}), (B) respiration rate, (C) mass specific growth rate, and (D) microbial carbon use efficiency (mCUE). Each box in the plot shows the upper and lower quartiles, with horizontal lines indicating the median values. The vertical lines represent the minimum and maximum values, and dots indicate outliers. The red dots represent the arithmetic mean. Different letters indicate statistically significant differences between the treatments and soils. Each box is calculated with a sample size of n = 5.

Soil respiration was significantly lower in Amhara than Sidama soils (p =<0.001). Lowest rates were recorded for Abiyot Fana soil, and highest for Bilate soil (Fig. 2B). Soil respiration was not related to plant DW, except for Kudmi soil in which C_{mic} correlated positively with plant DW (p = 0.010) (Fig. 3B). Statistically, soil respiration was unaffected by plant composition, but there was a significant difference between the two plant treatments in Abiyot Fana, with soil from the *Urochloa* × *Desmodium* mixture having a lower respiration rate than the *Urochloa* single stand (p = 0.010).

3.3 Mass specific growth rate and mCUE

Values of fDNA varied between the soils, with Abiyot Fana having the highest and Kudmi the

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lowest amount of C_{mic} per unit DNA extracted (Fig. 4), indicating differences in DNA extraction efficiency. However, these differences could not explain differences in mass specific growth rates which were significantly smaller in Amhara than Sidama soils ($p = <0.001$) (Fig. 2C). There was no correlation between plant DW and mass specific growth rate in either soil (Fig. 3C). Plant composition had a significant effect, with *Urochloa* in single stand tending to support larger mass specific growth rates ($p = 0.005$), which was most pronounced in Sidama soils. However, this effect was significant in Bilate soil only ($p = 0.032$).

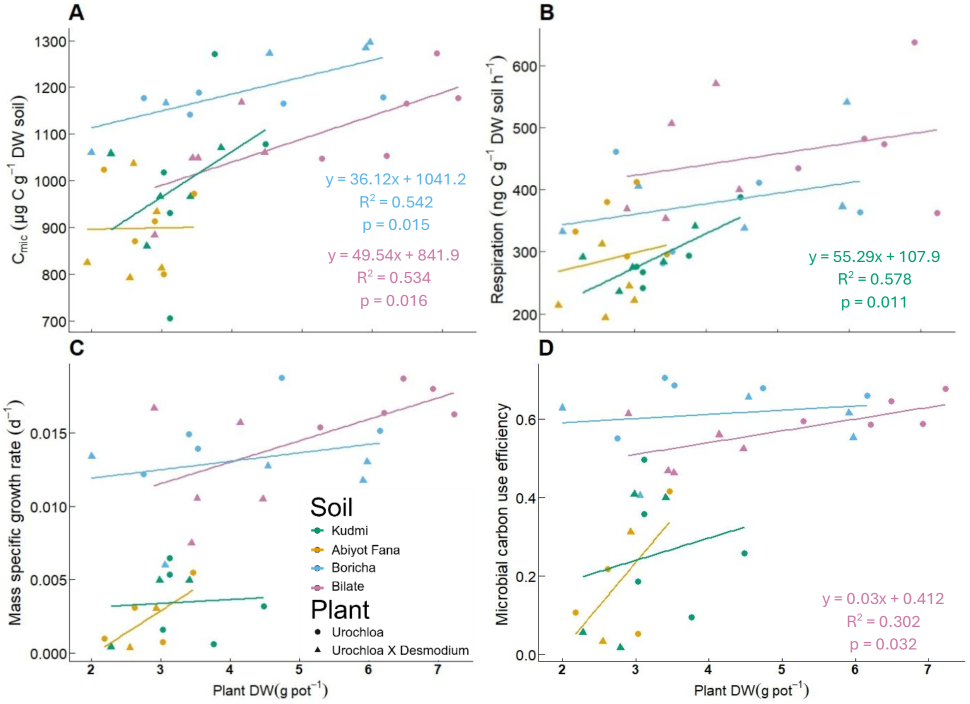

Figure 3. Linear regressions of (A) microbial biomass C (C_{mic}), (B) respiration, (C) mass specific growth rate and (D) microbial carbon use efficiency (mCUE) with plant dry weight (DW) in the four soils.

The mean mCUE of the four soils was 0.25 ± 0.17 in Kudmi soil, 0.19 ± 0.15 in Abiyot Fana soil, 0.61 ± 0.09 in Boricha soil and 0.57 ± 0.07 in Bilate soil (Fig. 2D). Microbial CUE varied significantly between the soils ($p = <0.001$), with lower values for Amhara than Sidama soils. Analyzed separately for each soil, mCUE was unaffected by plant DW, except for Bilate soil in which mCUE increased significantly with plant DW ($p = 0.032$) (Fig. 3D). The mCUE did not show any significant difference with plant composition in Kudmi, Abiyot

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Fana and Boricha soil. In Bilate soils, mCUE was significantly higher in *Urochloa* single stands than in mixtures ($p = 0.025$). A similar trend was observed in Boricha soil. We were unsuccessful in determining mCUE in the four control soils without plants, as ^{18}O incorporation was indiscernible from the non-labelled samples in all four soils, resulting in partly negative mCUE values. The control soils were therefore omitted from the statistical analyses.

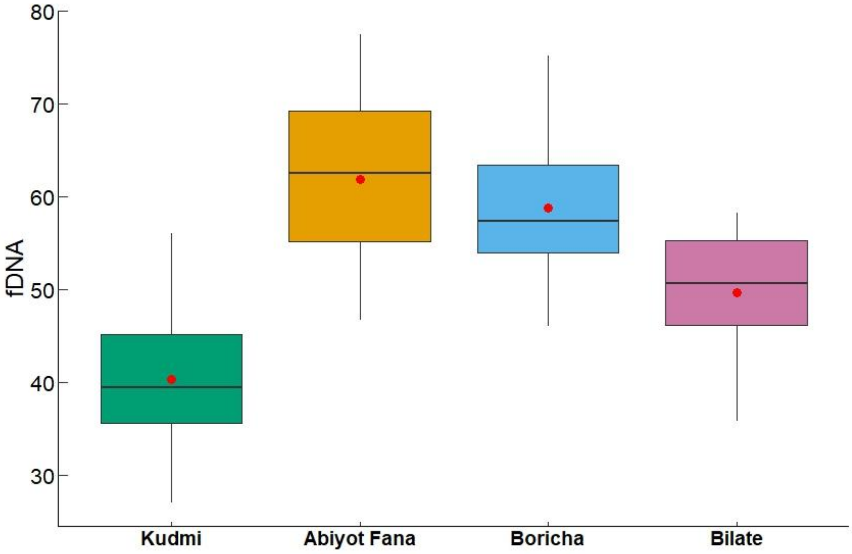

Figure 4. The conversion factor fDNA (C_{mic}/DNA) for the four soils. Different letters indicate statistically significant differences between the treatments. Each box in the plot shows the upper and lower quartiles, with horizontal lines indicating the median values and the red dot the arithmetic mean. Each box is calculated with a sample size of n = 10 (5 replicate pots and two technical replications).

4. Discussion

4.1 Soil specific variations in plant growth, microbial growth and mCUE

Initial soil properties affected plant growth performance, with plants grown in Sidama soils accumulating more biomass compared to plants in Amhara soils (Fig. 1). This difference reflects inherent differences in soil nutrient content; Sidama soils are less clay-rich, have a higher pH and hold more extractable P than Amhara soils (Table 1), making them the more fertile soils included in our study. We did not measure root biomass but the higher plant growth in Sidama soils may have increased root volume and length and hence the root-soil interface, making microbial communities in the Sidama soils more responsive to plant C inputs.

Organic C and total N contents were similar across the soils, as were their C:N ratios (Table 1). It is therefore unlikely that microbial responses differed because of differences in soil parental C and N availability. The single most striking difference between the soils from the two regions was the difference in Melich III extractable phosphorous (P), which was 11 - 16 times higher in Sidama than Amhara soils. A larger microbial P availability in Sidama soils may thus be key for a more balanced microbial nutrient status that would allow for more microbial growth in response to plant C input resulting in larger mCUE values (Wickander et al., 2026). Interestingly, differences in soil respiration (Fig. 2B) between the two regions appeared smaller than those for mCUE or mass specific growth rate (Fig. 2C), suggesting that mCUE across all four soils was more controlled by microbial growth than microbial activity.

Differences in soil texture may contribute to explaining the observed differences in mCUE. Amhara soils have higher clay and aluminum (Al) contents than Sidama soils. Clay may negatively impact microbial growth as it protects SOC from microbial attack so that more energy must be invested in extracellular enzyme synthesis which reduces both microbial growth and mCUE (Sinsabaugh et al., 2013). Others works reported higher mCUE in clay rich soils attributing this to more stabilization of microbial decomposition products and microbial necromass, which potentially lowers respiration (Angst et al., 2021; Cotrufo et al., 2013; Zhou et al., 2023). Our findings contradict this notion and point towards growth inhibition rather than reduced respiration as a regulating mechanism for mCUE in degraded Rift valley soils. In Amhara soils, additional factors may have reduced mCUE, such as low soil pH and Al toxicity (Jones et al., 2019).

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4.2 Effect of plant growth and mixture on microbial growth and mCUE

We confirmed our first hypothesis that mCUE response to plant input would depend on initial soil properties. In Amhara soils, plant composition had no effect on mass specific microbial growth or mCUE (Fig. 2C, D). This suggests that the realized plant C and N input did not alleviate nutrient deficiencies in these soils. Mass specific growth rates remained small, suggesting that the ability of soil microbial communities to respond to plant C and N input by growth in Amhara soils was stoichiometrically constrained, most likely by their severe P deficiency (Cleveland and Liptzin, 2007; Manzoni et al., 2012). Conversely, in the more fertile Sidama soils, which has significantly higher mCUE values, we observed that mCUE was notably lower in the *Urochloa* × *Desmodium* mixture than in the grass single stand. Although this effect was significant in the Bilate soil only, we observed a similar tendency in the Boricha soil. This refutes our hypothesis that N input from legumes in the mixture would increase mCUE. N input from legumes typically enhances microbial growth and activity (Poeplau et al., 2019; Spohn et al., 2016), but this may not necessarily result in higher microbial C uptake with increased mCUE in degraded, P-limited soils. Moreover, the N input from legumes and the low C:N ratio in legume litter can potentially increase decomposition rates (Kuzyakov, 2010; Manzoni et al., 2012) by promoting copiotroph microbes which tend to have low mCUE (Malik et al., 2019). However, we did not observe higher respiration rates in *Urochloa* × *Desmodium* mixtures compared with grass single stands.

Several indirect effects of legumes may have added to the finding of lower mCUE in mixtures. Generally, legumes exude less photosynthetically derived C to the soil than non-legumes owing to the high energy demand of symbiotic N-fixation. Once soil N increases due to the presence of legumes, plant C allocation to roots and exudates is downregulated as more native soil N is available. This results in less C available for soil microbes, which may decrease decomposition rates and microbial growth (Prommer et al., 2020). This would be consistent with our observation of reduced mass specific growth rates in the *Urochloa* × *Desmodium* mixtures in Sidama soils.

Together, this highlights stoichiometric controls as a strong driver for mCUE. Nutrient imbalances restrict microbial growth and incorporation of C into microbial biomass. In nutrient limited soils, microbes must therefore allocate more resources toward acquiring scarce nutrients. In a companion study with the same soils, we found increased investment in exoenzyme synthesis in the low N and P Amhara soils (Wickander et al., 2026), which could explain the low mCUE of these soils observed here. In general, more plant C must be invested in maintenance metabolism in nutrient poor soils (Poeplau et al., 2019; Spohn et al., 2016), which is consistent with the low mCUE values found for Amhara soils in the present study. Enhanced utilization of plant C for maintenance respiration in Amhara soils is also supported by the low mass specific growth rates (Fig. 2C) and high C_{mic}-specific respiration relative to amount of C_{mic}, both of which result in low mCUE.

4.3 Plant-soil interactions and mCUE

We could not confirm our second and third hypotheses that legume incorporation and plant diversity would increase mCUE; neither inclusion of legumes, nor plant biomass exhibited significant effects on mCUE, except for Bilate soil. While increased species diversity is well documented to increase soil C (Fornara and Tilman, 2008; Fry et al., 2026), our experiment has only two plant treatments (single stand and a mixture) grown over a 104-day period, which may not be sufficient to produce discernable effects on mCUE. Additionally, due to the failed ^{18}O-incorporation in microbial DNA extracted from unplanted controls, we could not analyze plant effect on mCUE *per se*. Yet, this failure demonstrates the pivotal role of plants for driving soil microbial activity and growth in degraded subtropical soils, even on the short term, and opens for plant-based soil remediation.

Notably, we found a suppressing effect of *Urochloa* × *Desmodium* mixture on mCUE, suggesting that plant functional types play a role. Previous studies have found that mCUE is not affected by species richness, even in long term experiments, but is instead affected by plant functional type (Prommer et al., 2020). Together with the strong dependency on soil nutrient status described above, this suggests that mCUE is controlled by both soil nutrient availability and plant C input. The significant correlation between plant biomass and mCUE, observed in Bilate soil suggests that biomass may act as a driving factor for high mCUE if P is available. When soil nutrient conditions support plant growth, plant C and N inputs can create a positive feedback by enhancing microbial growth and mineralization, which in turn supports plant growth and stabilization of plant C as SOC with multiple benefits for soil health.

5. Conclusions

This study investigates the effects of grass single stands and grass-legume mixture on mCUE across four weathered Ethiopian soils. Our results show that mCUE varies greatly with regional soil differences, while functional plant types play a minor role. Notably, mCUE was nearly twice as high in Sidama soils, which were characterized by higher pH and P availability, supporting increased microbial growth and mCUE. Overall, the regulation of mCUE appeared to depend on microbial growth rather than metabolism. This highlights the pivotal role homeostatic nutrient supply has on microbial growth and in consequence on mCUE and implies that highly degraded sub-Saharan soils must be phosphorous amended before using forage plants in a targeted way to ameliorate soils.

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Author contributions

NJW performed the greenhouse experiment and carried out laboratory work, processed and analyzed the data and wrote the initial draft. PD conceived the study together with NJW and assisted in data interpretation and writing.

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Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Data availability

The datasets generated during and/or analyzed under the current study are available at zenodo.org, https://doi.org/10.5281/zenodo.19821240.

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