Plant Soil (2026) 524:1099–1117
https://doi.org/10.1007/s11104-026-08705-2

RESEARCH ARTICLE

# Mulching, rhizobial inoculation, and water supply shape soil enzyme activities and nutrient dynamics in a Namibian sandy soil under cowpea cultivation

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Received: 10 February 2026 / Accepted: 26 May 2026 / Published online: 11 June 2026
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**Abstract**

*Background and aims* Agricultural soils in semi-arid sub-Saharan Africa are often low in soil organic carbon (SOC) and nitrogen (N), making them vulnerable to degradation and threatening food security of smallholder farmers. Conservation agriculture practices such as mulching are promoted as climate-resilient strategies to improve soil fertility, but it remains unclear whether these practices improve soil biogeochemical properties in semi-arid regions.

*Methods* We conducted a greenhouse experiment that simulated a cowpea (*Vigna unguiculata*) growth period, a regional key crop, under water optimum and stress conditions using sandy soil from the Omusati region in Namibia. Effects of different mulches (wheat straw, wood chips, 1:1 mixture), inoculation with a locally adapted *Bradyrhizobium* and water conditions on soil biogeochemical properties, including SOC, N, microbial biomass and enzyme kinetics (β-glucosidase, chitinase, leucine-aminopeptidase) were investigated.

*Results* We showed that mulching had limited short-term effects on SOC and N. Inoculation with rhizobia increased soil N, lowered C:N ratios and reduced N-related enzyme activities, demonstrating its potential to mitigate N limitation. Water stress decreased enzyme activities by 64%, indicating that water availability may outweigh management effects. Inoculation shifted the microbial demand from N towards C under water optimum, whereas all soils were N limited under water stress, reflected in enzymatic C:N ratios.

*Conclusion* Our findings highlight cowpea inoculation as an effective strategy to improve soil N, while mulching provides additional benefits for soil fertility only under favourable water conditions. Increased C and N contents show that combining inoculated

Responsible Editor: George N. Furey.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11104-026-08705-2.

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legumes with mulching can enhance nutrient availability in semi-arid agroecosystems, contributing to sustainable land management.

**Keywords** Low-fertility soils · C and N cycling · Legume cultivation · Climate-resilient agriculture · Greenhouse experiment

## Introduction

About one-third of soils in sub-Saharan Africa are degraded (Okolo et al. 2020) and have lost much of their soil organic carbon (SOC), total nitrogen (TN) and biological activity due to intensive agricultural use (Shiimi and Uchezuba 2025; Soropa et al. 2019; Srivastava et al. 2025). In sub-Saharan Africa, especially Namibia, the majority of arable land is under rainfed smallholder farming (Shiimi and Uchezuba 2025), but sandy, low-nutrient soils and harsh climatic conditions threaten food security (Amelung et al. 2020; Araya et al. 2024; Thierfelder and Mhlanga 2022). To overcome declining soil fertility and improve soil properties, low-cost and sustainable solutions are required (Chianu et al. 2011; Mutengwa et al. 2023).

Conservation agriculture (CA) is increasingly recommended for sustainable soil fertility management (Chapungu and Zhanda 2025; Thierfelder and Mhlanga 2022). Conservation agriculture is based on the three principles: minimum soil disturbance, permanent soil cover and crop rotation (FAO 2025). In semi-arid regions such as northern Namibia, where rainfall is erratic and droughts are frequent, CA can conserve water, regulate soil temperature (Masete et al. 2022; Mupangwa et al. 2012), and create favourable conditions for microbial activity (Masete et al. 2022), compared to traditional agriculture practices without soil cover.

Mulching has been reported as the most important of the three CA principles (Masete et al. 2022). As organic mulches improve soil nutrient cycling, microbial activity, and organic matter content during decomposition (Araya et al. 2024; El-Beltagi et al. 2022; Thierfelder and Mhlanga 2022), their effectiveness still depends on mulch type (El-Beltagi et al. 2022). However, consistent results of studies that investigate effects of mulch on low-nutrient sandy soils are still lacking (Chapungu and Zhanda 2025; Colunga et al. 2025). In addition, organic mulch materials are often scarce due to low crop yields and competition with feed for livestock (Araya et al. 2024; Chapungu and Zhanda 2025; Masete et al. 2022; Parwada et al. 2022). At the same time, bush clearing efforts to control shrub encroachment of various species generate large amounts of woody biomass, particularly from *Acacia* sp., which could provide a locally alternative mulch source in Namibia (Devine et al. 2017; Mupangwa et al. 2023; Zimmer et al. 2024).

To address soil N limitations, which are major constraints in sandy and degraded soils (de Blécourt et al. 2019), integrating legumes into CA has generally pronounced benefits (Soropa et al. 2019; Thierfelder and Mhlanga 2022). Legumes can restore soil N due to their ability to fix dinitrogen from the atmosphere through a mutualistic symbiosis with rhizobia (Dakora and Phillips 2002; El Sabagh et al. 2020; Gogoi et al. 2018). Legumes also contribute high-quality organic matter (OM) and root exudates that improve nutrient availability for following crops (Becker et al. 2024; Hansen et al. 2017; Siddique et al. 2012). However, their ability to fix N is restrained in drought-prone regions of north-eastern Namibia, as nodulation is often reduced by water stress and heat (Grönemeyer et al. 2014; Taylor et al. 2021). To improve nodulation and provide a sufficient number of effective rhizobia, inoculation with locally adapted rhizobia can be used as a low-cost and environmentally friendly biofertilizer (Chen et al. 2024; Deaker et al. 2004; Figiel et al. 2025; Lupwayi et al. 2011; Rasche et al. 2023). In addition, inoculation with locally adapted rhizobia supports legume growth, leading to higher plant biomass and thus improved crop yields as a result of higher nutrient availability as well as increased drought tolerance through enhanced plant growth and nodulation (Abd-Alla et al. 2023; Chen et al. 2024; Laishram et al. 2024; Wang et al. 2025a). However, it remains unclear how soil water availability, mulching and inoculation interact to influence soil chemical properties.

Management practices influence soil microbial activity (Zuber and Villamil 2016). Microbial parameters, such as microbial biomass and extracellular enzyme activities, are key indicators of change in soil quality, as they respond quickly to changes (Adetunji et al. 2017; Malobane et al. 2020). Extracellular enzymes, released by roots and microorganisms,

such as β-glucosidase (BG), chitinase (N-acetylglucosaminidase; NAG), and leucine-aminopeptidase (LAP), mediate soil organic matter (SOM) decomposition and nutrient mobilisation (Burns et al. 2013; Jiang et al. 2025; Kuzyakov and Razavi 2019; Liu et al. 2024b; Razavi et al. 2016b) and respond sensitively to changes in soil moisture and temperature caused by mulch (Ndossi et al. 2020; Song et al. 2024). As enzyme activities directly reflect microbial access to C and N, enzymatic ratios can further indicate whether soil processes are C- or N-limited (Sinsabaugh et al. 2008). In sandy soils with low OM, microorganisms may respond more sensitively to mulch application and substrate availability, leading to stronger changes in enzyme activity than in soils with higher soil fertility and OM content (Islam et al. 2025; Tian et al. 2020), yet detailed studies under semi-arid conditions like northern Namibia are lacking.

It remains unclear how soil water availability, mulch and inoculation interact to influence soil chemical and microbiological properties. Thus, we investigated their combined effects on soil chemical and microbiological properties in low-nutrient sandy soils of northern Namibia. We conducted a greenhouse experiment simulating a crop season, in which cowpeas were grown in these soils under two soil water conditions, with different mulch types and inoculation. Soil chemical and microbiological properties were assessed, including SOC, N pools, microbial biomass, pH, and enzyme activities. We hypothesised that mulch application 1) increases SOC as well as TN content and 2) enhances enzyme activities due to a more favourable environment for microorganisms, that 3) rhizobial inoculation increases N availability in the soil through enhanced plant growth, and 4) the combination of mulch and inoculant further amplifies these responses, while 5) mulch buffers negative effects of water stress on these biogeochemical soil properties.

## Material and methods

### Study area

The experiment was conducted using mixed topsoil (0–10) samples from the Ogongo campus of the University of Namibia (UNAM) in the Omusati region (17°41′08.9"S 15°18′01.1" E). The site had an irregular land-use history and remained fallow prior to sampling. Prior to sampling, the site was ploughed and weed harvested. This region has a semi-arid climate and represents a major agricultural region in Northern Namibia. The soil was sampled in January 2023, air-dried, sieved (< 2 mm) and homogenised. Afterwards the soil was stored in plastic boxes until further use for soil property assessment and experimental start. The soil has a sandy texture (87% sand content), and initial soil properties of 0.30% SOC, a TN content of 0.026% and a pH ($CaCl_2$) of 5.38.

### Experimental design

We conducted the experiment from May to July 2024 in a greenhouse at the University of Hamburg, Germany, using pots (height 12 cm, diameter 10 cm) filled with the sandy soil from the Omusati region (Fig. S1). Experimental conditions were controlled and aimed to simulate semi-arid growing conditions of day and night temperatures of 30 °C and 15 °C, with a relative humidity of 50%. A natural daylight cycle of 12 h (6:00–18:00) was attained through natural daylight and with additional illumination via plant lights when necessary.

The experiment followed a factorial design combining two soil water levels: water optimum (45% of maximum water holding capacity) and water stress condition (20% of maximum water holding capacity) with four mulch treatments: no mulch (to represent traditional low-input agriculture), plus *Acacia* sp. wood chips (wood), plus wheat straw (straw), and plus a 1:1 combination of both (wood + straw) (Fig. 1). Mulch types differed in substrate quality, whereas wood mulch has a lower C:N ratio with 61.30 compared to straw with a C:N ratio of 97.17. Mulch treatments were designed to simulate CA conditions and were applied to fully cover the soil surface. Additionally, pots were either inoculated with 1 ml (corresponding to $2 \times 10^8$ cells) of a locally adapted *Bradyrhizobium* sp. strain 26-nodO (sequence of 16S-23S ribosomal RNA intergenic spacer region, GenBank PX914841) with Modified Arabinose Gluconate medium, in liquid culture, directly after sowing (referred to as inoculated) or left uninoculated but treated with 1 ml of sterile liquid Modified Arabinose Gluconate medium (referred to as planted). The rhizobial strain had been isolated from nodules of the cowpea variety Bira grown at the

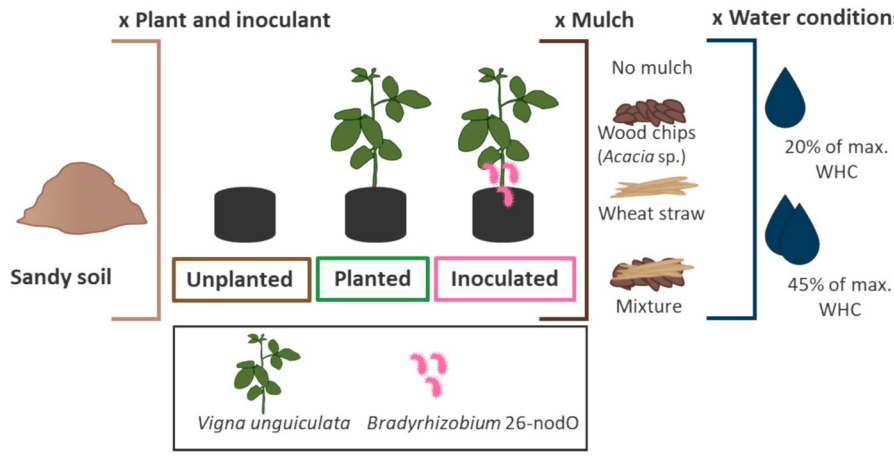



**Fig. 1** Experimental setup to investigate mulch, cowpea inoculation and their interaction effects under different water conditions. Unplanted = pots without cowpea, planted = pots planted with cowpea, inoculated = pots with inoculated cowpea, max. WHC = maximum water holding capacity

UNAM campus in Ogongo at a neighboring site (A. Sarkar and B. Reinhold-Hurek, unpublished results). Unplanted pots, per water level and mulch treatment, served as the control. Each treatment consisted of four replicates, and treatment-specific water content was ensured by weighing and watering each pot every five days to simulate fluctuations in water availability. These fluctuations allowed for drying phases between watering, which, taking water dynamics into account, reflected differences in evaporation caused by mulch application. Consequently, mulched treatments received a lower total amount of water compared to unmulched treatments. Cowpea (*Vigna unguiculata*; variety Lutembwe), a common legume in southern Africa, was grown until pod initiation. Germinated seedlings were provided by the Department of Microbe-Plant Interactions of the University of Bremen. Before planting, cowpea seeds were surface-sterilised in freshly prepared 2.5% sodium hypochlorite for six minutes, washed repeatedly with sterile distilled water and then placed on 1% water agar plates to germinate for 24–30 h at 30 °C in dark to prevent seedling diseases. Plants were fertilised with 100 kg $ha^{-1}$ granulated superphosphate per pot to avoid phosphorus deficiency (Becker et al. 2024).

## Sampling and analyses

Sampling of plants was done when more than 50% of water optimum plants reached pod initiation after 12 weeks. Then, soil and plants were sampled destructively. After separation of plants and soil, soil samples were homogenised, sieved < 2 mm and frozen until further laboratory analyses. To determine plant aboveground and belowground biomass, roots were washed and together with shoots of respective plants dried at 60 °C for 72 h and measured as drymass.

Soil pH was measured in a 1:2.5 soil-to-$CaCl_2$ ratio (MP230 pH meter, Mettler Toledo). Soil carbon and nitrogen were analysed on milled and 105 °C oven-dried samples by dry combustion using an elemental analyser (varioMAX cube, Elementar Analysensysteme GmbH, Langenselbold, Germany). Since pH was below 7, carbonates were not expected, and we assumed that total carbon was equivalent to SOC.

For $N_{min}$ (ammonium and nitrate) soil was shaken for one hour with 0.0125 M $CaCl_2$ in a 1:4 soil-to-solution ratio. Ammonium was analysed photometrically at 655 nm. To determine nitrate, the same extract was analysed by high-performance liquid chromatography (Agilent Binary Technologies 1200 Series, Santa Clara, CA, USA).

Microbial biomass was determined using the chloroform fumigation method (Vance et al. 1987). The fresh soil samples (12.5 g dry weight equivalent) (watered at 40% of max WHC) were extracted in a 1:4 ratio of 0.05 M $K_2SO_4$. Another 12.5 g dry weight equivalent of fresh soil was fumigated with chloroform in a desiccator for 24 h and then extracted. Afterwards, the extracts were used to analyse dissolved organic C and N content in a 1:2 dilution with $K_2SO_4$ with a TOC/TN Analyser (Shimadzu Corporation, Kyoto, Japan). $C_{mic}$ calculations were based on the difference between $K_2SO_4$-extractable C and N in fumigated and non-fumigated soils, using the conversion factors of 0.45 for $C_{mic}$ (Joergensen 1996).

## Enzyme assays

Activities of the extracellular enzymes were determined using synthetic fluorogenic substrates either with 4-methylumbelliferon (MUF) or 7-amido-4-methylcoumarin (AMC), followed by the protocol of Marx et al. (2001). The fluorogenic substrate used to detect β-glucosidase (BG) was MUF-β-glucopyranoside, for chitinase (NAG) it was MUF-N-acetyl-ß-D-glucosaminide, and for leucine-aminopeptidase (LAP) it was L-leucine-AMC-hydrochloride. All substrates and chemicals were purchased from Sigma-Aldrich (Darmstadt, Germany). For the soil suspension, fresh (4 °C) soil samples (0.5 g dry weight equivalent) were weighed in sterile 100 ml glasses, and 50 ml of sterile MilliQ water was added. Then, the soil solution was sonicated for 2 min with an output energy of 40 $J^{-1}$. Afterwards, aliquots of 50 µl were pipetted into sterile deep-well plates (Nunc™ F96 MicroWell™ plate, black, 96 well, 238 Thermo Scientific™). Next, 50 µl of 0.1 M MES for MUF linked substrates or 0.05 M TRIZMA buffer for AMC linked substrates and 100 µl of substrate solution (0, 10, 20, 30, 40, 50, 100, and 200 µmol $g^{-1}$) were added. Three analytical replicates were measured for each sample at each substrate concentration. Fluorescence was measured by a microplate reader (Victor$^3$ 1420–050 Multi label Counter, PerkinElmer, USA; extinction: 355 nm, emission: 460 nm) immediately after substrate addition (t0), 30 min (t1), 1 h (t2), and 2 h after addition (t3) under controlled temperature of 22 °C. For calibration and accounting for quenching, standard plates were prepared with 50 µl of a composite soil solution (for each treatment), with 150, 140, 130, 100, 70, and 30 µl of buffer (MES or TRIZMA) and 0, 10, 20, 50, 80, and 120 µl standard (MUF or AMC), respectively. The soil enzyme activity was quantified by calibrating with the regression slopes of the standard measurements, enzyme activities of the samples were calculated [nmol substrate $g^{-1}$ soil $h^{-1}$]. Activities were fitted by the Michaelis–Menten Equation (Eq. 1; Michaelis and Menten (1913)), which describes nonlinear saturation curves and the catalytic enzyme efficiency was calculated after Moscatelli et al. (2012) Eq. 2:

$$v = \frac{V_{max} * [S]}{K_m + [S]} \quad (1)$$

$$K_a = \frac{Vmax}{K_m} \quad (2)$$

with $v$ as the reaction rate of the respective soil enzyme at a certain substrate concentration [nmol substrate $g^{-1}$ soil $h^{-1}$], $V_{max}$ as the maximal rate of enzymatic activity at the given temperature, [S] as the substrate concentration (µM), $K_m$ as the half-saturation constant as indicator for substrate affinity and $K_a$ as the catalytic enzyme efficiency. Higher $K_a$ shows better catalytic properties (Moscatelli et al. 2012).

Extracellular enzyme stoichiometry (Eq. 3) was calculated as the ratio of C- (BG), and the sum of N-related (NAG + LAP) enzymes after Sinsabaugh et al. (2008) and the description of Moorhead et al. (2016), Liu et al. (2024a) and Yang et al. (2020) as follows:

$$\text{Soil enzyme C:N ratio} = \frac{\ln\left(V_{max}BG\right)}{\ln\left(V_{max}NAG + V_{max}LAP\right)} \quad (3)$$

The soil enzymatic C:N ratio of C-and N-acquiring enzymes can be used to reflect soil microbial nutrient demand of C versus N. While a ratio of C:N of 1:1 would represent an equilibrated system, values > 1 indicate C limitation.

## Statistical analyses

All calculations and statistical analyses were done using RStudio (Version 4.5.0) using 'dplyr' package for data handling (Hadley et al. 2023), 'stats' package (R Core Team 2025) for statistical analyses, 'multcomp' (Hothorn et al. 2008) package for compact letter display, 'outliers' (Komsta 2022) package to detect outliers, and 'ggplot2' (Wickham 2016) package for data visualisation. To test for significant differences ($p < 0.05$) within one water condition of $V_{max}$, $K_m$, and $K_a$, respectively, we used two-way ANOVA (with plant and mulch as factors) with Tukey HSD post-hoc test for pairwise comparison. To estimate effects of water conditions, we used t-test to compare each treatment between water conditions to allow a more robust statistical design. Outliers of $V_{max}$, $K_m$, and $K_a$ were detected using Dixon's Q test to check the measurement

accuracy. To test whether the enzymatic C:N ratio differs statistically from one a one-sampled t-test was used. To test for significant differences of SOC, TN, SOC:N ratio and $C_{mic}$, respectively, we used the same statistical analyses and p-values $\leq 0.1$ were declared as tendencies of significance.

## Results

### Soil chemical and microbiological properties

Under water-optimum conditions, in the presence of plants and inoculated plants SOC increased, while inoculation tended to increase TN. The C:N

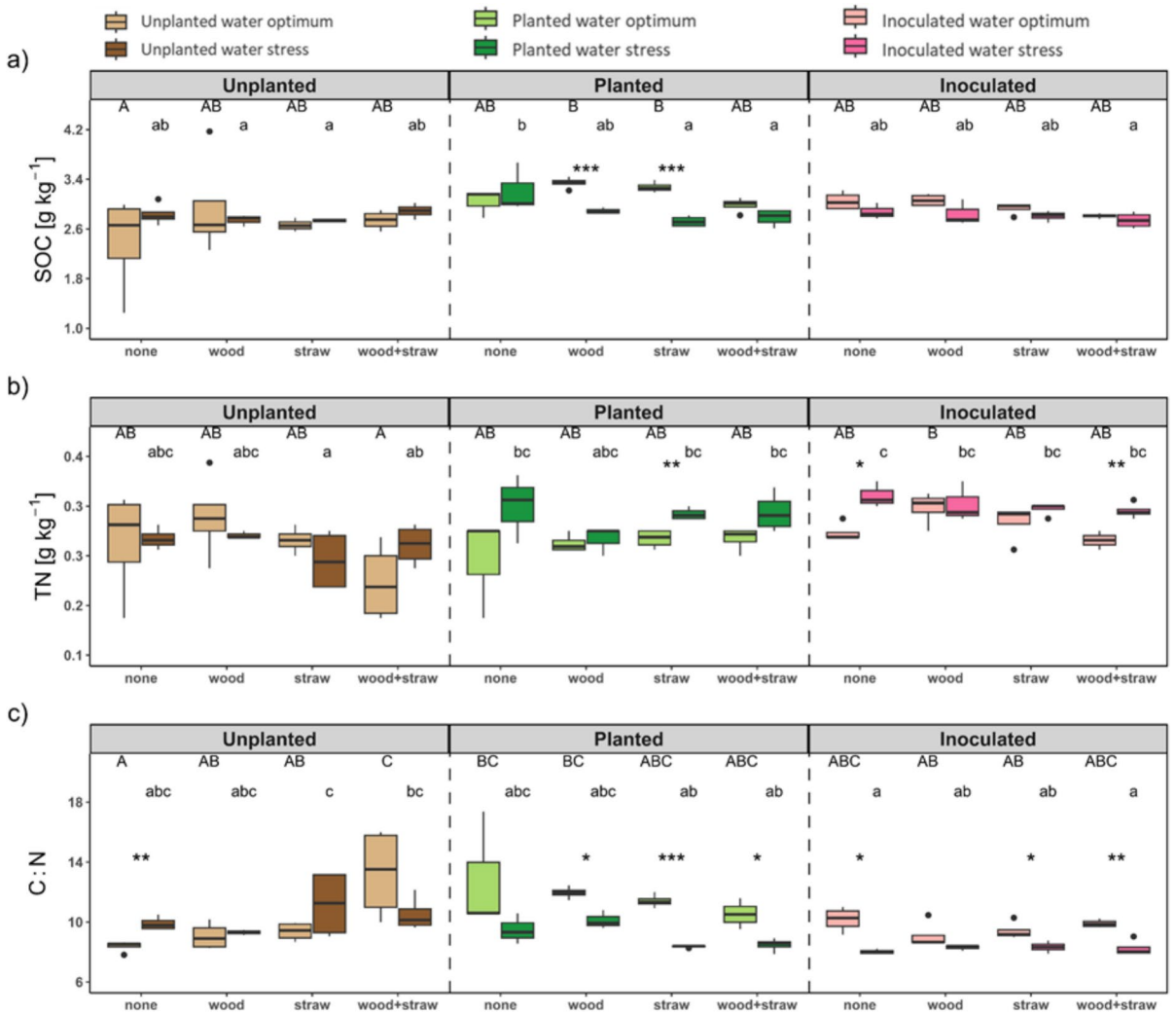



**Fig. 2** **a**) Soil organic carbon (SOC) content (g kg$^{-1}$), **b**) total nitrogen (TN) content (g kg.$^{-1}$), and **c**) soil C:N ratio in unplanted, planted and inoculated treatments ($n=4$) with applications of no mulch, wood chips (wood), wheat straw (straw) and a combination of wood chips and wheat straw (wood + straw). Different letters indicate significant differences according to Tukey's HSD test, where capital letters show significant differences within water optimum condition and small letters within water stress condition. In addition, asterisks indicate significant differences within one treatment between different water conditions, whereas number of asterisks indicate the level of significance (*** $p<0.001$, ** $p<0.01$, * $p<0.05$)

ratio decreased with inoculation and the interaction between plant, inoculation and mulch under both water conditions (Table S1). Under water stress conditions, SOC was decreased by mulch, whereas TN was increased by plant and inoculation (Table S1).

The SOC content was approximately 20% higher in planted treatments with wood and straw application under water optimum compared to water stress conditions (Fig. 2a). These treatments also showed higher SOC contents than the unplanted unmulched treatment. Under water optimum conditions, TN was higher in inoculated ($0.31 \pm 0.007$ g $kg^{-1}$) compared to planted ($0.279 \pm 0.009$ g $kg^{-1}$) treatments (Fig. 2b). Under water stress, TN increased in the planted treatment with straw (+14%), in the unmulched inoculated (+20%) and in the inoculated wood + straw treatment (+17%) compared to water optimum conditions ($p < 0.05$; Fig. 2b). The soil C:N ratio decreased under water stress in planted treatments with wood (−18%), straw (−36%) and wood + straw (−24%) compared to water optimum conditions ($p < 0.05$; Fig. 2c). Similar decreases were observed in the inoculated unmulched (−27%), straw (−13%) and wood + straw (−20%) treatments ($p < 0.05$). Inoculation tended to lower the C:N ratio under both water conditions ($p < 0.05$; Fig. 2c).

Under water optimum conditions, $C_{mic}$ generally decreased with mulch addition, whereas under water stress conditions, plant and inoculation, as well as their interaction with mulch, mostly decreased $C_{mic}$ (Table S1).

Particularly, with straw and wood + straw mulch in the planted treatment, $C_{mic}$ decreased ($p < 0.05$; Fig. 3). Under water stress conditions, inoculation tended to decrease $C_{mic}$, whereas wood mulch increased it ($p < 0.05$). The highest $C_{mic}$ were observed in planted treatments without mulch (+50%) and with wood addition (77%) under water stress conditions.

Mineral N was highest in the unplanted compared to planted and inoculated treatments (Fig. S3). No differences were observed in the planted and inoculated treatments under both water conditions. The root dry weight correlated with the C and N content of the root under water optimum conditions, whereas no other soil parameter correlated with the root dry weight under both water conditions (Fig. S4).

## Soil enzyme kinetics

Across all enzymes, $V_{max}$ of BG and NAG was approximately ten times higher than that of LAP (Fig. 4). Under water optimum conditions, plant, inoculation and their interaction with mulch increased $V_{max}$ of BG, whereas LAP was affected by plant and inoculation as well as mulch. No treatment effects were observed for NAG (Table S2).

Under water optimum conditions, $V_{max}$ of BG was higher in inoculated treatments (without mulch

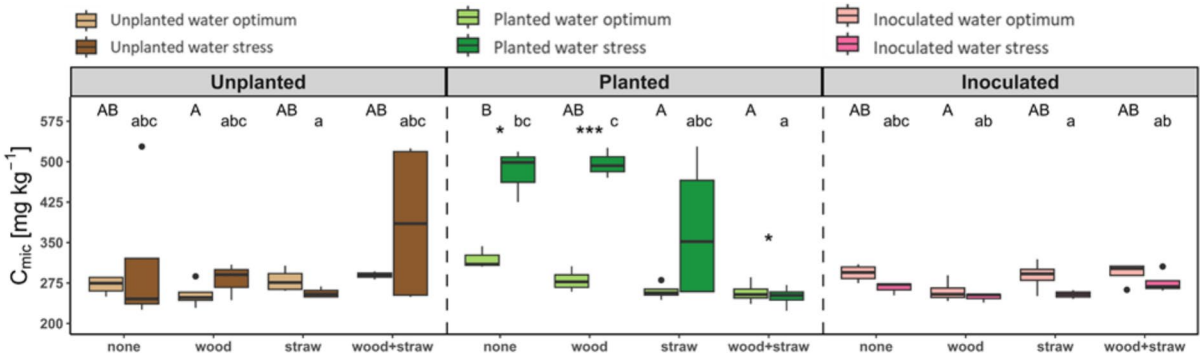



**Fig. 3** Microbial biomass carbon ($C_{mic}$) in unplanted, planted and inoculated treatments (n = 4) with applications of no mulch, wood chips (wood), wheat straw (straw) and a combination of wood chips and wheat straw (wood + straw).. Different letters indicate significant differences according to Tukey's HSD test, where capital letters show significant differences within water optimum condition and small letters within water stress condition. In addition, asterisks indicate significant differences within one treatment between different water conditions, whereas number of asterisks indicate the level of significance (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$)

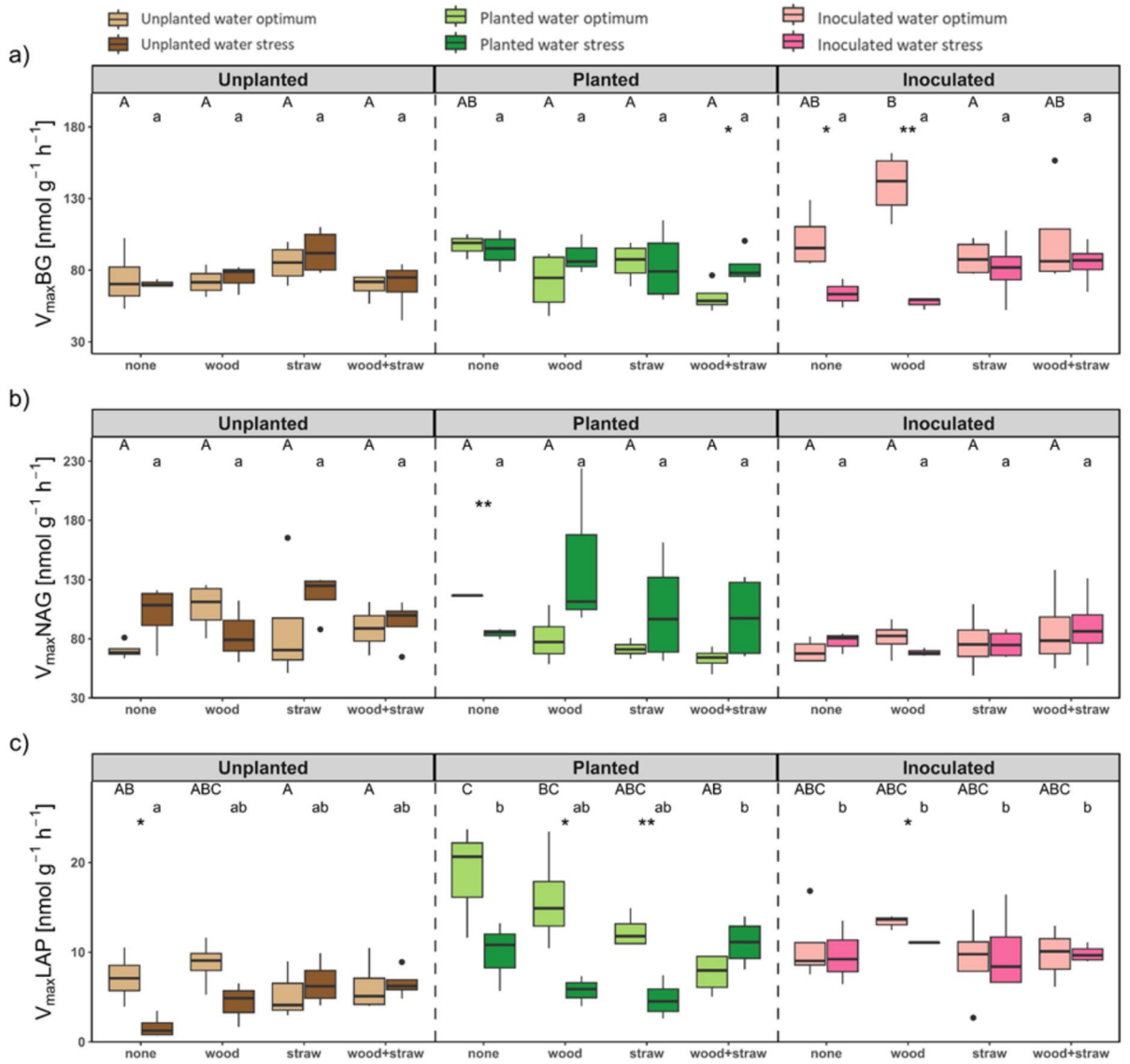



**Fig. 4** **a**) Enzyme activity $V_{max}$ of ß-glucosidase (BG), **b**) chitinase (NAG), and **c**) leucine-aminopeptidase (LAP) in unplanted, planted and inoculated treatments ($n=4$) with applications of no mulch, wood chips (wood), wheat straw (straw) and a combination of wood chips and wheat straw (wood + straw). Different letters indicate significant differences according to Tukey's HSD test, where capital letters show significant differences within water optimum condition and small letters within water stress condition. In addition, asterisks indicate significant differences within one treatment between different water conditions, whereas number of asterisks indicate the level of significance (*** $p<0.001$, ** $p<0.01$, * $p<0.05$)

and with wood addition) compared to the other treatments ($p<0.05$; Fig. 4a). Under water optimum conditions $V_{max}$ of BG was up to 143% higher than under water stress conditions for inoculated and wood mulched treatments, with the combination of wood and inoculation showing the highest activity ($140 \pm 22.8$ nmol g$^{-1}$ h$^{-1}$). For $V_{max}$ of NAG, no water-related effects occurred, only unmulched planted treatment was up to 38% higher under water optimum than water stress conditions (Fig. 4b). Under both water conditions, $V_{max}$ of LAP decreased with mulch (Fig. 4c). Overall, $V_{max}$

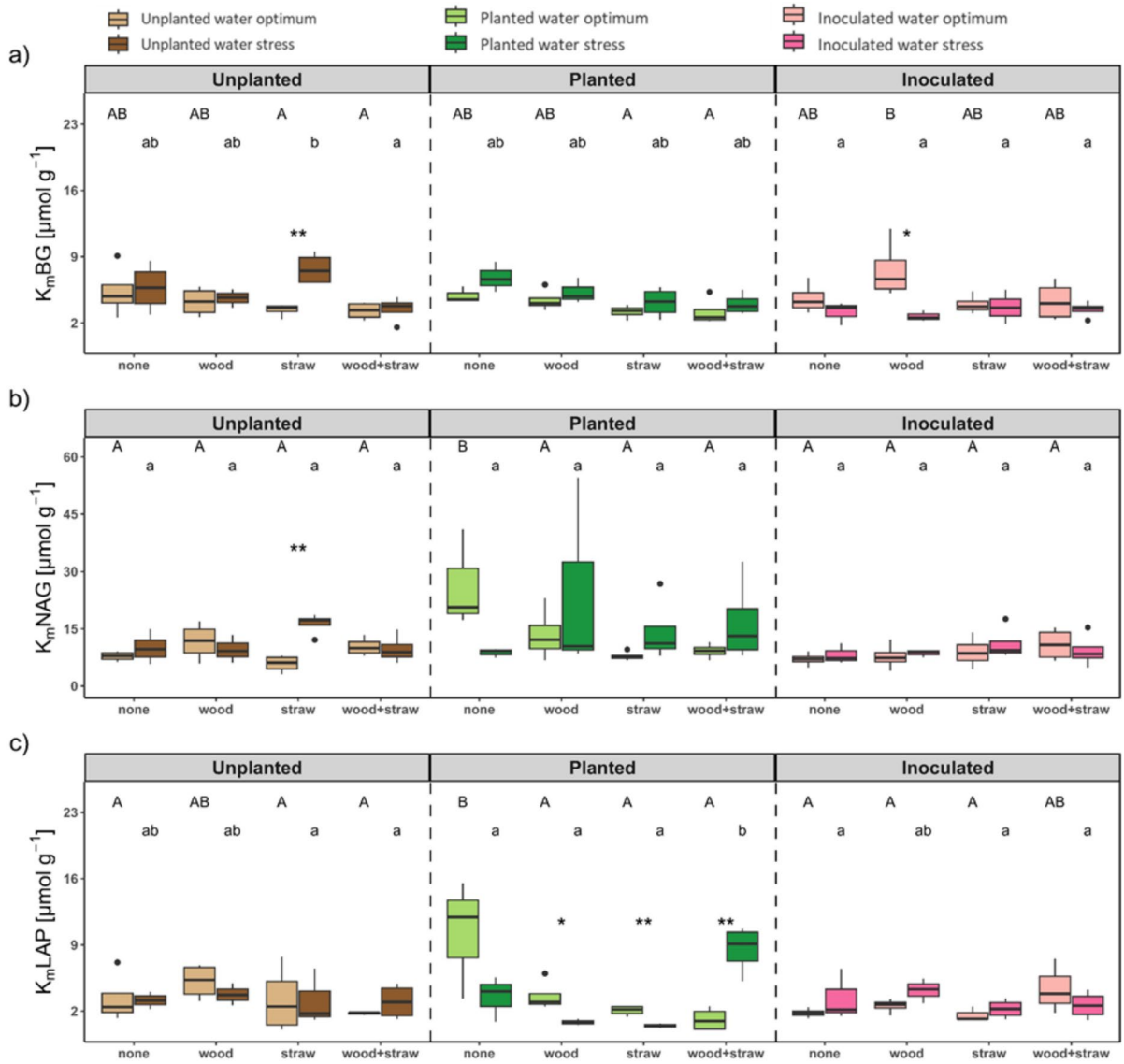



**Fig. 5** **a**) Enzyme affinity $K_m$ of ß-glucosidase (BG), **b**) chitinase (NAG), and **c**) leucine-aminopeptidase (LAP) in unplanted, planted and inoculated treatments ($n=4$) with applications of no mulch, wood chips (wood), wheat straw (straw) and a combination of wood chips and wheat straw (wood + straw). Different letters indicate significant differences according to Tukey's HSD test, where capital letters show significant differences within water optimum condition and small letters within water stress condition. In addition, asterisks indicate significant differences within one treatment between different water conditions, whereas number of asterisks indicate the level of significance (*** $p<0.001$, ** $p<0.01$, * $p<0.05$)

of LAP was highest in planted treatments and under water optimum conditions for the unmulched, wood (+178%) and straw (+159%) mulched treatments. For the inoculated treatment, only wood application differed significantly between water optimum and water stress conditions.

Across all enzymes, $K_m$ for NAG was approximately three times higher than that of BG and LAP (Fig. 5), whereas high $K_m$ values reflect low substrate affinity. Under water optimum conditions, mulch affected $K_m$ of BG and LAP, whereas also their interaction affected $K_m$ of LAP (Table S3).

No treatment effects were observed for NAG (Table S3). Treatment effects were less pronounced under water stress conditions, only $K_m$ of BG and LAP was decreased by plant and inoculation and mulch.

Under water stress conditions, $K_m$ of BG was lower in the inoculated wood (+181%) treatment compared to water optimum conditions, while mulch increased $K_m$ under water optimum conditions ($p < 0.05$; Fig. 5a). Under water stress conditions, $K_m$ of BG was lower in inoculated treatments. For NAG, except the unplanted straw treatment, where $K_m$ of NAG was up to 179% higher under water stress conditions no effects were observed (Fig. 5b). Mulch decreased $K_m$ of LAP under both water conditions, except the planted wood + straw and inoculated wood treatments under water stress conditions ($p < 0.05$; Fig. 5c). Overall, the influence of mulch and inoculation on $K_m$ was more pronounced for LAP and BG under water optimum, whereas water stress reduced these effects.

Under water optimum conditions, mulch increased $K_a$ of BG, and plant and inoculation affected $K_a$ of NAG and LAP (Table S4). Under water stress conditions, $K_a$ of BG was influenced by plant and inoculation, while $K_a$ of NAG decreased and $K_a$ of LAP increased with mulch. In addition, LAP was affected by plant and inoculation and the interaction with mulch (Table S4).

Under water optimum conditions, mulch increased $K_a$ of BG ($p < 0.05$; Table S4). Water stress reduced $K_a$ of BG only in the unplanted straw (−53%) and planted treatment without mulch (−31%; Fig. 6a). For $K_a$ of NAG under water optimum conditions, an increase in the unplanted straw treatment compared to the planted unmulched, planted wood and planted wood + straw treatments was observed ($p < 0.05$; Fig. 6b). For LAP, mulch increased $K_a$ only under water stress conditions, for the planted straw treatment ($p < 0.05$; Fig. 6c).

Overall, mulch increased $K_a$ of BG under water optimum conditions and $K_a$ of LAP under water stress conditions (Table S4).

Soil enzymatic C:N ratios revealed that under water optimum conditions, unplanted (wood, wood + straw) and planted treatments (no mulch, wood + straw) were N limited (Fig. 7). In contrast, inoculated treatments (no mulch, wood) were C limited. Under water stress conditions, the C:N balance shifted, and all treatments were N-limited.

## Discussion

### Limited mulch effects on soil organic matter and enzyme kinetics

Contrary to our hypothesis, mulch applications did not increase SOC and TN contents. Similar results have been reported in other studies, where SOC significantly increased only after mulching for more than one vegetation period, as short-term residue inputs were insufficient to alter bulk soil nutrient pools (Noor ul et al. 2022; Ortega et al. 2023; Rossi et al. 2024; Sapkota et al. 2015). A field experiment with cowpeas conducted by Sapkota et al. (2015) also did not observe significant increases in OM and N content after the first mulch application. Similarly, SOC and TN did not differ between mulched and unmulched soils under both water conditions, likely due to the short 84-day duration of our experiment (Ortega et al. 2023; Rossi et al. 2024). This might be attributed to the slow decomposition of the applied mulch under laboratory conditions. In contrast, under field conditions, decomposers such as termites may accelerate OM turnover and enhance soil N at later stages (Giller et al. 2009; Njoroge et al. 2022; Xu et al. 2024).

Similar effects of straw and wood mulches on SOC and TN indicate that mulch quality was less important in short-term SOM and nutrient turnover (Rossi et al. 2024). Such short-term effects are supported by previous findings from Ortega et al. (2023), who reported no differences in total C between wood and straw mulch after 6 months after a wildfire in a forest with a sandy loam, suggesting limited residue impact on SOC across different soil types and ecosystems. Although mulch type did not affect soil chemical properties under the laboratory conditions, wood mulch may still be important to protect against wind erosion, rainsplash and surface runoff (Amiri Ghanat Saman et al. 2025; Appiah et al. 2023) in sandy soils under field conditions.

Although mulch did not alter SOC and TN after one vegetation period, it can still influence microbial processes (Akhtar et al. 2018). Our hypothesis that

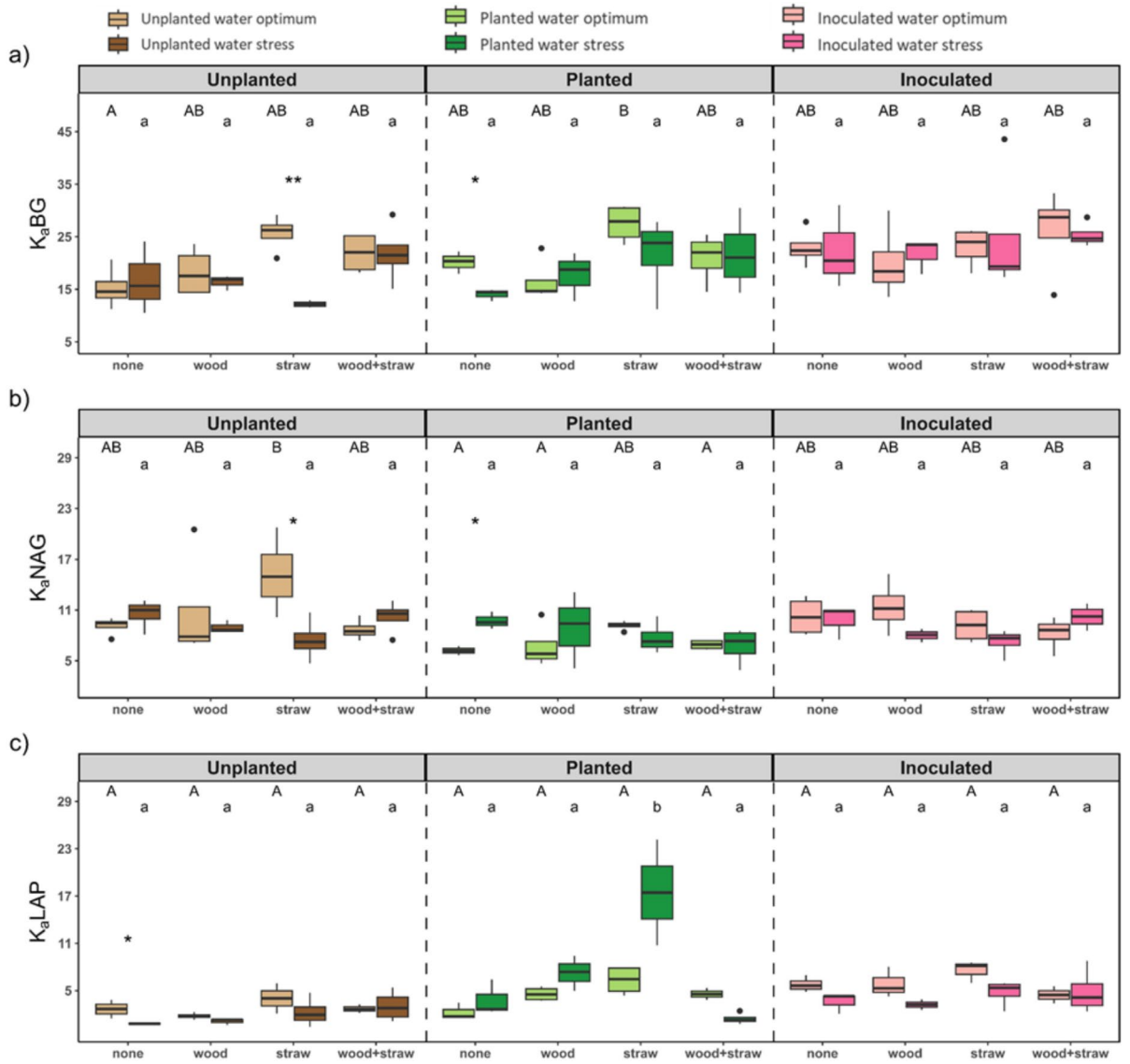



**Fig. 6** **a**) Catalytic efficiency $K_a$ of ß-glucosidase (BG), **b**) chitinase (NAG), and **c**) leucine-aminopeptidase (LAP) in unplanted, planted and inoculated treatments ($n=4$) with applications of no mulch, wood chips (wood), wheat straw (straw) and a combination of wood chips and wheat straw (wood + straw). Different letters indicate significant differences according to Tukey's HSD test, where capital letters show significant differences within water optimum condition and small letters within water stress condition. In addition, asterisks indicate significant differences within one treatment between different water conditions, whereas number of asterisks indicate the level of significance (*** $p<0.001$, ** $p<0.01$, * $p<0.05$)

enzyme activities would increase under mulch application was only partially supported. The type and quality of mulch determine whether they primarily supply C or also contribute to N availability, which affects microbial nutrient cycling. Straw mulch, with its high C:N ratio, provides predominantly labile C upon decomposition, which can temporarily stimulate microbial growth but can temporarily induce N immobilization, as microorganisms assimilate available mineral N to meet their demands (El-Beltagi et al. 2022; Kader et al. 2017; Masciandaro et al. 2004; Rossi et al. 2024; Uwituze et al. 2023). In contrast, wood chip mulches, like *Acacia* wood chips, with its comparatively lower C:N ratio may supply both C and

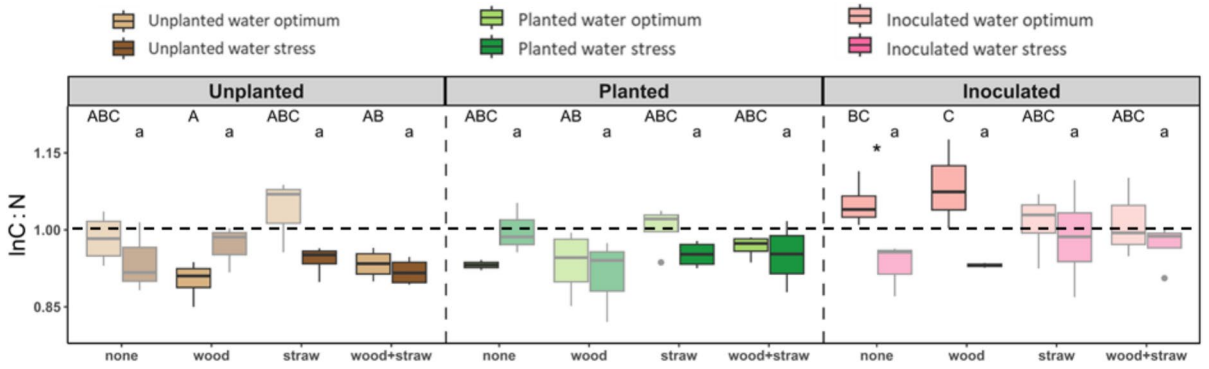



**Fig. 7** Enzymatic ratios of C:N (lnBG:ln(NAG + LAP)) in unplanted, planted and inoculated treatments ($n = 4$) with applications of no mulch, wood chips (wood), wheat straw (straw) and a combination of wood chips and wheat straw (wood + straw). Different letters indicate significant differences according to Tukey's HSD test, where capital letters show significant differences within water optimum condition and small letters within water stress condition. In addition, asterisks indicate significant differences within one treatment between different water conditions, whereas number of asterisks indicate the level of significance (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$) and unshaded boxplots indicate significant differences from 1 according to t-test

N during decomposition, potentially reducing microbial N limitation and shifting resource allocation strategies. Our results revealed that mulch alone did not increase BG activities in unplanted and planted soils, but likely supplied labile substrates that supported microbial growth and nutrient turnover (Akhtar et al. 2019; Du et al. 2022; Moscatelli et al. 2012; Noor ul et al. 2022; Wang et al. 2025b). Since microbial biomass ($C_{mic}$) reflects changes in soil management and soil resource availability (Adetunji et al. 2017; Burns et al. 2013; Malobane et al. 2020), larger microbial communities can be seen as an indicator of greater enzyme synthesis resulting in higher enzyme activities (Akhtar et al. 2019; Gianfreda 2015; Qu et al. 2023), whereas in general enzyme production is controlled by substrate availability and microbial nutrient demand (Allison et al. 2011; Burns et al. 2013). Our results show higher LAP activity and lower substrate affinity (high $K_m$) in planted unmulched soils compared to planted mulched and inoculated soils suggesting microorganisms responded to substrate scarcity in unmulched soils by upregulating enzyme production rather than improving enzyme efficiency (Allison and Vitousek 2005; Razavi et al. 2016a; Stock et al. 2019; Stone et al. 2012). The half-saturation constant $K_m$ is an indicator for the affinity of the enzyme for its substrate and is temperature dependent (Moscatelli et al. 2012). Mulch influences biological activity not only through substrate supply and its quality but also through modification of the soil microclimate, including water conservation and temperature regulation (Araya et al. 2024; El-Beltagi et al. 2022; Guja and Bedeke 2024), which in turn affects microbial and enzymatic activity (Allison et al. 2010; Jafarian et al. 2023; Razavi et al. 2016a). Since enzyme activity ($V_{max}$) is more temperature sensitive than substrate affinity ($K_m$) (Razavi et al. 2016a), supposedly higher soil temperatures in unmulched soils likely enhanced microbial activity and thus enzyme production (Qu et al. 2023), explaining increased LAP enzyme activity. Thus, the observed difference in LAP enzyme activities between mulched and unmulched soils reflect shifts in soil microclimate that shaped enzyme production and efficiency. Further, enzymatic C:N ratios in unplanted and planted soils with mulch (wood, wood + straw) indicated higher BG activity relative to N-related enzyme activity, suggesting that short-term organic inputs preferentially stimulate microbial C acquisition rather than N turnover (Noor ul et al. 2022; Uwituze et al. 2023). This suggests that microorganisms remained N limited despite increased C availability. Although organic mulches often stimulate microbial metabolism and enzyme efficiency (Jiang et al. 2025; Noor ul et al. 2022; Ortega et al. 2023; Wang et al. 2025b), this effect was less pronounced in our study, suggesting that C-acquiring enzyme production depends not only on C but also on N availability.

### Improved nitrogen turnover driven by inoculated legumes

Cowpea inoculation, with a locally adapted *Bradyrhizobium* strain from the specific agricultural site in Namibia, tended to increase soil N dynamics, supporting our hypothesis that legumes improve N availability in nutrient-poor sandy soils. Inoculated soils showed higher TN content and lower soil C:N ratios than uninoculated soils. This reflects enhanced N fixation and N inputs as well as rhizodeposition by inoculated cowpeas (Fustec et al. 2010; Pausch and Kuzyakov 2018; Srivastava et al. 2025). In planted soils, rhizodeposition further modulates microbial substrate availability, as plant roots continuously release labile C compounds into the rhizosphere, stimulating microbial activity (Pausch and Kuzyakov 2018; Zhu et al. 2014). Rhizodeposits of legume crops or inoculated legume crops additionally include N-rich compounds derived from symbiotic $N_2$ fixation, which can locally increase N (Kebede 2021; Siddique et al. 2012; Thilakarathna et al. 2016; Zhu et al. 2014) and can stimulate the microbial and enzymatic activity (Srivastava et al. 2025; Thilakarathna et al. 2016). Thus, increased microbial N availability suggests that improved N fixation through legume inoculation can balance N limitations under our experimental conditions (Masete et al. 2022). Further, reduced LAP enzyme activity in inoculated soils underlines this, implying that microbial N demand declines due to inoculated legumes and increased legume-rhizobial symbiosis (Virk et al. 2022). This finding is supported by shifts in enzymatic C:N ratios. Uninoculated soils were N-limited, whereas inoculated soils tended to be C-limited. This demonstrated increased microbial N availability through legume inputs (Kanté et al. 2023; Liu et al. 2025; Siczek et al. 2018) and shifted limitation towards C (Allison et al. 2011; Tharanath et al. 2024), and their interaction response might result in a balanced C and N demand.

### Combined effects of mulch application and legume inoculation

As mulch can increase—mainly C—substrate availability (Akhtar et al. 2018) and inoculation enhances N inputs through legume-rhizobia symbiosis (Wang et al. 2025a), their interaction was expected to influence enzyme activity and nutrient cycling (Chen et al. 2021). The enzyme activity increased only under combined inoculation and mulching, indicating interactions between biological N inputs and substrate supply as well as quality. The activity of BG was highest in inoculated wood mulched soils, while other mulched or planted soils showed no effect. This likely reflects both the higher wood mulch quality indicated by its lower C:N ratio (Kader et al. 2017; Rossi et al. 2024) compared to straw mulch in our study and the increased N availability through inoculation. This was especially the case under water optimum conditions that accelerated the positive synergetic effect. Accordingly, higher BG activity in inoculated wood mulched soils likely demonstrates microbial responses to combined availability of labile C and N, as TN can stimulate BG activity (Allison and Vitousek 2005; Jiang et al. 2025; Stock et al. 2019).

### Low water availability restricting treatment effects

While mulch and inoculation affected microbial and nutrient dynamics, their effectiveness depended on water availability. Under water optimum conditions, SOC was about 20% higher in some mulched soils (wood, straw) than under water stress conditions, suggesting that mulch effects on soil chemical parameters are more pronounced when sufficient water is available. This is likely explained by enhanced root growth and exudation that accelerates microbial organic carbon turnover resulting in C stabilisation (Deng et al. 2021; Liang 2020; Qu et al. 2023; Srivastava et al. 2025; Wang et al. 2025b). While TN was unaffected by mulching, individual mulch types in uninoculated soil (wood, straw) increased TN by up to 20% under water stress compared to water optimum conditions. This increase might be likely due to reduced microbial activity and N plant uptake resulting in a drought-driven accumulation of N (Deng et al. 2021; Leitner et al. 2017; Thilakarathna et al. 2016; Zhu et al. 2018). Moreover, higher TN under water stress conditions in inoculated unmulched and combined mulched soils may reflect increased N inputs through rhizodeposition from symbiotic $N_2$ fixation. Such increases in TN following biological $N_2$ fixation are well documented in literature (Becker et al. 2024; Ortiz and Wolf 2024; Thilakarathna et al. 2016), where plant growth and associated rhizodeposition contribute to soil N accumulation. Therefore,

enhanced TN in inoculated treatments is likely related to improved $N_2$ fixation resulting from successful inoculation, rather than reduced plant N uptake. This suggests that inoculated legume crops may be less dependent on water-mediated mass flow of N to the root compared to uninoculated plants. This aligns with studies underlining this finding, reporting that inoculation can enhance soil N under drought and improve plant drought tolerance through maintained photosynthetic activity (Álvarez-Aragón et al. 2023; Jabborova et al. 2021; Wang et al. 2025a). Nevertheless, to verify this assumption, isotopic methods such as $^{15}N$ natural abundance methods would be required.

Enzymatic responses to water availability differed between enzymes. Water stress, mainly decreased LAP and partly BG activities, indicating that soil moisture limits microbial activity and nutrient turnover in our soil (Bogati and Walczak 2022; Deng et al. 2021; Qu et al. 2023), and that LAP is more drought-sensitive than BG. This is further reflected in a decrease in enzymatic C:N ratios and reduced enzyme activity of LAP under water stress, where all soils were N-limited. Further demonstrating that water availability controls microbial nutrient dynamics (Qu et al. 2023; Wang et al. 2025b).

## Conclusion

Our study suggests that mulching during one vegetation period alone has limited effects on soil C and N dynamics in sandy soils of northern Namibia cultivated with cowpea under greenhouse conditions. It was only effective under favourable water conditions. In contrast, legume inoculation with locally adapted rhizobia consistently enhanced TN contents and lowered C:N ratios, highlighting its potential to improve soil fertility even under water-limited conditions. Enzyme kinetics indicated that inoculation reduced the microbial N demand under water optimum conditions and shifted towards a C limitation. In addition, microbes can partially adapt to water stress by increasing substrate affinity, although enzyme activities declined and nutrient turnover was limited. These findings demonstrated the effectiveness of sustaining soil fertility when legume inoculation is combined with practices such as mulching, a core principle of CA. While we did not observe additional short-term effects of *Acacia* wood chips compared to other mulch types, they could serve as an effective option for common mulching materials, especially in areas with soil erosion danger and limited mulch availability. Overall, integrating legumes with organic mulches represents a promising strategy to enhance soil biogeochemical properties and nutrient availability in semi-arid systems.

**Acknowledgements** We thank Jacob Kohn, Deborah Harms, Annika Naumann, Sumita Rui, Maria Seedtke (University of Hamburg) and Moses Ngugi (University of Tuebingen) for help with laboratory work and the Institute of Plant Sciences and Microbiology, Department Biology, University of Hamburg for the use of greenhouse and Maren Winnacker for constant support. Further, we thank Callum Banfield (University of Tuebingen) for support in laboratory issues.

**Author contributions** Annette Eschenbach, Joscha N. Becker, Simran Sekhri and Elisa Karina Albrecht contributed to the study conception and design. Material preparation and data collection were done by Elisa Karina Albrecht and Simran Sekhri. Data analysis was performed by Elisa Karina Albrecht with methodological contributions from Svenja C. Stock and Joscha N. Becker. The first draft of the manuscript was written by Elisa Karina Albrecht and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

**Funding** Open Access funding enabled and organized by Projekt DEAL. This work was funded by the Federal Ministry of Research, Technology and Space, Germany, in the framework of SASSCAL 2.0 (grant no. 01LG2051B to AE and 01LG2051A to BRH), as well as the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy, EXC 2037 CLICCS Climate, Climatic Change, and Society (Project number: 390683824), contribution to the Centre for Earth System Research and Sustainability (CEN) of the University of Hamburg.

**Data availability** The data is available upon request from the corresponding author.

**Declarations**

**Generative AI and AI-assisted technologies in the writing process** Statement: During the preparation of this work, the author(s) used DeepL and Grammarly to improve the readability and language of the manuscript. After using this tool, the author(s) reviewed and edited the content as needed and take full responsibility for the content of the published article.

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

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