The Author

Early on, Elisa Karina Albrecht was interested in applying scientific knowledge to address environmental challenges. She therefore studied Geosciences with a focus on Soil Science during her Bachelor's and Master's studies at the University of Hamburg. During this time, she developed an interest in field work and soil processes. Building on this background, she continued her academic path with a doctorate in the working group Soil Protection at the department of Earth System Sciences at the University of Hamburg. Her doctoral research focused on improving soil fertility through legume cultivation and conservation agriculture in low-fertility soils in Namibia.

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Impact of legumes on soil nutrient distribution, carbon and nitrogen cycling with regard to soil restoration in the Kavango region, Namibia

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submitted by

Elisa Karina Albrecht

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Abstract

Land degradation and climatic changes threaten soil fertility and thus smallholder farmers in sub-Saharan Africa. Conservation agriculture (CA) and legume cultivation are considered potential strategies for improving soil fertility. Legume cultivation can improve soil nitrogen status, particularly in the rhizosphere, through nitrogen fixation and root exudation. A better understanding of the processes and mechanisms linking soil, plants and microorganisms is required to assess their effectiveness in low-fertility sandy soils.

Sub-Saharan Africa is projected to be a climate change hotspot. Two drought-prone regions in northern Namibia were selected as exemplary regions to investigate how CA and legume cultivation can enhance soil fertility. The effects of CA and legume cultivation on soil carbon and nitrogen, as well as on microbiological parameters, were examined through field and laboratory experiments. For all experiments, sandy soils from Namibia were used, and cowpea (*Vigna unguiculata*) served as an exemplary legume crop. To analyse the impact of land management, a field study was conducted in the Kavango region with three land-use types: CA, traditional agriculture (TA) and woodland (W), with W representing near-natural conditions. Soil samples were collected from two depths and analysed for pH, texture, organic carbon and nitrogen content, plant-available phosphorus, cation exchange capacity, microbial biomass and microbial community.

In addition, a greenhouse experiment using sandy soil from the Omusati region simulated a growing season under different management practices (CA and TA), with *Bradyrhizobium* sp. inoculation to potentially enhance nitrogen fixation and with uninoculated soil, under high and low soil water availability. Effects on soil chemical and microbial parameters were assessed by measuring soil organic carbon and nitrogen, pH, microbial biomass and enzyme kinetic parameters. To investigate mechanisms of legume cultivation on carbon and nitrogen dynamics during cowpea growth and the influence of nodule senescence, a laboratory rhizobox experiment was conducted. Soil organic carbon and nitrogen content were determined, and *in situ* zymography was used to visualise the spatial distribution of enzyme activities in the soil at three plant growth stages.

The effects of land management and legumes on soil properties varied across temporal and spatial scales. After six years of CA in the field, no improvements in soil chemical properties compared to TA were observed. Conservation agricultural management did not improve the soil chemical properties to W level. In contrast, microbial diversity, expressed as Shannon-

Wiener and Simpson indices, showed significantly higher diversity under CA compared to W, with a slight trend towards higher microbial diversity under CA compared to TA. This upward trend in microbial diversity was reflected in calculated potential crop yields, which tended to be 26% higher under CA compared to TA. The greenhouse experiment further demonstrated the absence of a short-term management effect on soil chemical properties, as mulching, one principle of CA, alone did not increase soil organic carbon. Only under optimum soil water availability, mulching resulted in higher soil organic carbon content compared to low water availability. However, the combination of mulching and inoculation proved particularly effective for nitrogen enrichment in soil. Across studies, microbial parameters responded more sensitively than soil chemical parameters. This was demonstrated in the greenhouse experiment, where carbon- and nitrogen-related enzyme activities responded differently to water availability and mulching. Furthermore, leucine-aminopeptidase, a nitrogen-related enzyme, responded more sensitively to low water availability, with reduced enzyme activity, than the carbon-related enzyme β-glucosidase. Mulching reduced leucine-aminopeptidase activity, while the combination of mulching and inoculation had the opposite effect on β-glucosidase. These differences in microbial responses in enzyme activities were mainly mediated by cowpea roots and the resulting nitrogen input. Further, the rhizobox experiment demonstrated that cowpea cultivation in sandy and loamy-sandy Namibian soils maintained high enzyme activities through root exudation and rhizodeposition during plant growth, irrespective of initial soil properties. In bulk soil, temporal and spatial responses differed: enzyme activities increased with plant growth but remained lower than in the rhizosphere. At maturity stage, nodule senescence provided an additional pulse of labile organic matter, resulting in increased enzyme activity.

In conclusion, the impact of legumes on nutrient dynamics of soils in the Kavango and Omusati regions in Namibia was mainly visible in the rhizosphere, creating local hotspots of carbon- and nitrogen-cycling. This might lead to gradual soil restoration. In combination with inoculation and inputs from decaying plant organs (nodules), soil organic matter may increase, thereby improving soil fertility. Field results indicate that under the prevailing climatic conditions, continuous inputs of organic mulch material – like *Acacia* wood chips originating from bush clearing – are necessary to balance nutrient losses through agricultural land use. Thus, soil improvement in northern Namibia can be achieved gradually through a combination of legume cultivation, inoculation and mulching. Further research is needed to understand how climate change will affect soil fertility of agricultural systems and how nodule senescence may influence legume-based systems.

Zusammenfassung

Bodendegradation und Klimawandel bedrohen die Bodenfruchtbarkeit und damit die Lebensgrundlage von Kleinbauern in Subsahara-Afrika. Konservierende Landwirtschaft (conservation agriculture, CA) und der Anbau von Leguminosen gelten als potenzielle Strategien zur Verbesserung der Bodenfruchtbarkeit. Vor allem der Anbau von Leguminosen kann den Stickstoffhaushalt des Bodens, insbesondere in der Rhizosphäre, durch biologische Stickstofffixierung und Wurzelexsudate erhöhen. Um die Wirksamkeit dieser Bodenmanagementpraktiken in nährstoffarmen, sandigen Böden beurteilen zu können, ist ein besseres Verständnis der zugrunde liegenden Prozesse und Mechanismen erforderlich, die Boden, Pflanzen und Mikroorganismen miteinander verbinden.

Da Subsahara-Afrika als Hotspot des Klimawandels gilt, wurden zwei dürregefährdete Regionen im Norden Namibias als exemplarische Untersuchungsgebiete ausgewählt, um zu untersuchen, wie CA und der Anbau von Leguminosen zur Verbesserung der Bodenfruchtbarkeit beitragen können. Die Effekte von CA und dem Anbau von Leguminosen auf den Kohlen- und Stickstoffhaushalt des Bodens sowie auf mikrobiologische Parameter wurden anhand von Feld- und Laborversuchen untersucht. In allen Experimenten wurden sandige Böden aus Namibia verwendet, wobei die Kuhbohne (*Vigna unguiculata*) als repräsentative Leguminose diente. Um zu untersuchen, wie sich verschiedene Landnutzungen und Bewirtschaftungspraktiken auf die Bodenfruchtbarkeit auswirken, wurde eine Feldstudie mit den drei Landnutzungstypen CA, traditionelle Landwirtschaft (traditional agriculture, TA) und Wald (W) in der Kavango-Region im Norden Namibias durchgeführt. Dabei repräsentierten W-Flächen naturnahe Bedingungen. Bodenproben wurden aus zwei Tiefen entnommen, und pH-Wert, Bodenart, organischer Kohlenstoffgehalt, Stickstoffgehalt, pflanzenverfügbarer Phosphor, Kationenaustauschkapazität sowie mikrobielle Biomasse und Gemeinschaften wurden analysiert.

Außerdem wurde ein Gewächshausexperiment mit sandigem Boden aus der Region Omusati durchgeführt, in dem eine Vegetationsperiode mit unterschiedlichen Bewirtschaftungspraktiken (CA und TA) sowie mit und ohne Zugabe von *Bradyrhizobium* sp. zur potenziellen Erhöhung der Stickstofffixierung bei hoher und niedriger Wasserverfügbarkeit simuliert wurde. Die Effekte auf chemische und mikrobielle Bodenparameter wurden anhand von Messungen des organischen Kohlenstoff- und Stickstoffgehalts, des pH-Wertes, der mikrobiellen Biomasse sowie der enzymkinetischen Parameter bewertet. Zusätzlich wurde ein Rhizobox-Experiment zur Untersuchung der Mechanismen und Effekte des Anbaus von Leguminosen auf die Kohlen- und Stickstoffdynamik während des

Pflanzenwachstums durchgeführt. Dafür wurden der organische Kohlenstoff- und Stickstoffgehalt und zusätzlich *in-situ* Zymographie, welche die räumliche Verteilung der Enzymaktivitäten visualisiert, an drei Pflanzenwachstumsstadien analysiert.

Die Auswirkungen von Bewirtschaftungspraktiken und dem Leguminosenanbau auf Bodeneigenschaften variierten in Abhängigkeit von räumlichen und zeitlichen Skalen. Nach sechs Jahren CA unter Feldbedingungen konnte im Vergleich zu TA keine Verbesserung der chemischen Bodeneigenschaften festgestellt werden. Konservierende Landwirtschaft führte nicht zu einer Verbesserung der chemischen Bodeneigenschaften auf das Niveau der Waldböden. Im Gegensatz dazu war die mikrobielle Diversität, ausgedrückt als Shannon-Wiener- und Simpson-Indizes, unter CA signifikant höher als im W, mit einem leichten Trend zu höherer Diversität unter CA im Vergleich zu TA. Dieser Anstieg der mikrobiellen Diversität spiegelte sich in berechneten potenziellen Erträgen wider, die unter CA um etwa 26% höher waren als unter TA (nicht statistisch signifikant). Auch im Gewächshausexperiment blieben kurzfristige Effekte der Bewirtschaftung auf die chemischen Bodeneigenschaften aus, da Mulch als Prinzip von CA den Gehalt an organischem Kohlenstoff im Boden nicht erhöhte. Lediglich bei optimaler Bodenwasserverfügbarkeit führte Mulchen zu höheren Kohlenstoffgehalten, während die Erhöhung bei geringer Bodenwasserverfügbarkeit ausblieb. Allerdings erwies sich die Kombination aus Mulchen und Inokulation als besonders effektiv bei der Anreicherung von Stickstoff im Boden. Über alle experimentellen Skalen hinweg reagierten mikrobielle Parameter empfindlicher als chemische. Dies zeigte sich vor allem in unterschiedlichen Reaktionen der kohlenstoff- und stickstoffbezogenen Enzymaktivitäten auf die Wasserverfügbarkeit und die Mulchzugabe. Leucine-Aminopeptidase, als stickstoffbezogenes Enzym, reagierte mit reduzierter Aktivität empfindlicher auf geringe Wasserverfügbarkeit als das kohlenstoffbezogene Enzym β-Glucosidase. Während Mulchen die Aktivität von Leucine-Aminopeptidase verringerte, zeigte die Kombination aus Mulch und Inokulat einen gegenteiligen Effekt auf die Aktivität von β-Glucosidase. Diese unterschiedlichen mikrobiellen Reaktionen wurden hauptsächlich durch den Anbau der Kuhbohne und den damit verbundenen Stickstoffeintrag bewirkt. Das Rhizobox-Experiment zeigte zusätzlich, dass der Anbau von Kuhbohnen in sandigen und lehmig-sandigen Böden Namibias während des Pflanzenwachstums hohe Enzymaktivitäten durch Wurzelexsudation und Rhizodeposition aufrechterhalten kann, unabhängig von den Bodeneigenschaften. Im Boden abseits der Rhizosphäre nahmen die Enzymaktivitäten mit fortschreitendem Pflanzenwachstum zu, wohingegen sie in der Rhizosphäre generell höher waren und konstant blieben. Im Reifestadium der Kuhbohne führte die Knöllchenseneszenz zu einem zusätzlichen Eintrag leicht verfügbarer organischer Substanz und damit zu erhöhter Enzymaktivität in unmittelbarer Nähe der seneszierten Knöllchen.

Zusammenfassend zeigte sich, dass die Auswirkungen von Leguminosen auf die Nährstoffdynamik der Böden in den namibischen Regionen Kavango und Omusati vor allem in der Rhizosphäre sichtbar waren, wo lokale Hotspots des Kohlen- und Stickstoffumsatzes entstanden. Dies kann zu einer schrittweisen lokalen Erhöhung der Bodenfruchtbarkeit beitragen. In Kombination mit Inokulat und organischen Einträgen, insbesondere Knöllchen, kann der Gehalt an organischer Bodensubstanz erhöht und damit die Bodenfruchtbarkeit verbessert werden. Die Ergebnisse der Feldstudie deuten darauf hin, dass unter den vorherrschenden klimatischen Bedingungen kontinuierliche Einträge von Mulchmaterial, beispielsweise aus Akazienholz, das aus der Bekämpfung der Verbuschung stammt, erforderlich sind, um Nährstoffverluste durch landwirtschaftliche Nutzung auszugleichen. Eine schrittweise Verbesserung der Böden im Norden Nambias kann somit durch eine Kombination aus Leguminosenanbau, Inokulation und Mulchen erreicht werden. Weitere Studien sind notwendig, um zu verstehen, wie sich der Klimawandel auf die Bodenfruchtbarkeit landwirtschaftlicher Systeme auswirkt und welche Rolle die Knöllchenseneszenz in leguminosenbasierten Anbausystemen spielt.

Manuscripts related to this dissertation

Manuscript A:

Elisa Karina Albrecht*, Joscha N. Becker, Judith Giel, Andrea Schaffrath, Barbara Reinhold-Hurek, Annette Eschenbach: Soil carbon, nitrogen and microbial responses under different land-use types in Northern Namibia

In preparation for submission

Elisa Karina Albrecht: Writing – original draft preparation, Investigation, Conceptualisation, Formal analysis and Investigation. Joscha N. Becker: Writing – review & editing, Conceptualisation, Methodology, Supervision. Judith Giel: Investigation, Writing – review & editing. Andrea Schaffrath: Investigation. Barbara Reinhold-Hurek: Writing – review & editing, Resources. Annette Eschenbach: Writing – Review & Editing, Conceptualisation, Supervision, Resources, Funding acquisition.

Manuscript B:

Elisa Karina Albrecht*, Simran Sekhri, Barbara Reinhold-Hurek, Shanmugam Solaiyappan Mani, Svenja C. Stock, Michaela A. Dippold, Annette Eschenbach, Joscha N. Becker: Mulching, rhizobial inoculation, and water supply shape soil enzyme activities and nutrient dynamics in a Namibian sandy soil under cowpea cultivation

Submitted

Elisa Karina Albrecht: Writing – original draft preparation, Investigation, Conceptualisation, Formal analysis and Investigation. Simran Sekhri: Investigation, Methodology, Writing – review & editing. Barbara Reinhold-Hurek: Writing - review & editing, Funding acquisition. Shanmugam: Writing - review & editing. Svenja Stock: Methodology, Writing – review & editing. Michaela Dippold: Writing – review & editing, Resources. Annette Eschenbach: Writing – Review & Editing, Supervision, Resources, Conceptualisation, Funding acquisition. Joscha N. Becker: Writing – review & editing, Conceptualisation, Methodology, Supervision.

Manuscript C:

Elisa Karina Albrecht*, Maire Holz, Joscha N. Becker: Spatio-temporal distribution of enzyme activities in cowpea rhizosphere – the role of plant growth stages and nodule senescence.

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Elisa Karina Albrecht: Writing – original draft, Investigation, Conceptualisation, Formal analysis. Maire Holz: Writing – Review & Editing, Resources, Methodology. Joscha N. Becker: Writing – Review & Editing, Conceptualisation, Methodology, Supervision.

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Further contributions to manuscripts

Simran Sekhri, **Elisa Karina Albrecht**, Alexander Gröngröft, Joscha N. Becker, Annette Eschenbach. 2025 (*accepted for publication in CABI, Special Issue, Soil Science*). Investigating soil water dynamics to improve agriculture: Namibian land-use systems under drought.

Simran Sekhri, **Elisa Karina Albrecht**, Jacob Kohn, Shanmugam Solaiyappan Mani, Barbara Reinhold-Hurek, Joscha N. Becker, Annette Eschenbach. *(submitted).* Cowpea growth response to mulching and inoculation modifying soil water balance under simulated drought.

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1 Introduction

1.1 Background and motivation

1.1.1 Soil degradation and fertility constraints in sub-Saharan Africa and Namibia

Soils are fundamental to ecosystem functioning and food production, providing essential services such as nutrient cycling, food security, carbon (C) sequestration and the maintenance of biological activity (LAL, 2015; LAL, 2016; KOPITTKE et al., 2019). However, approximately one-third of the world's soils have been degraded through intensive agriculture, organic matter (OM) depletion and land-use change (LAL, 2015; AMELUNG et al., 2020; THIERFELDER & MHLANGA, 2022; SHIIMI & UCHEZUBA, 2025; SRIVASTAVA et al., 2025).

Across sub-Saharan Africa (SSA), the dominance of subsistence farming under rainfed conditions makes soils particularly vulnerable to degradation, directly affecting agricultural productivity and livelihoods (CHIANU et al., 2011; AMELUNG et al., 2020; KIRSTEN et al., 2024; ABSHIBA et al., 2025). In many regions of SSA, soils are sandy, which limits their water and nutrient retention capacity, and have soil organic carbon (SOC) contents below 0.5% limiting agricultural productivity (HARTEMINK & HUTING, 2008; THIERFELDER et al., 2015; HUANG & HARTEMINK, 2020; RÖTTER et al., 2024). Rapid population growth further intensifies these problems, as Africa's population is projected to double by 2050, with the majority living in SSA (KOPITTKE et al., 2019; NATIONS, 2021; OSEWE et al., 2023; MUTANGA et al., 2024). The growing demand for arable land, combined with poor soil fertility, poses a major threat to regional food security (HARTEMINK & HUTING, 2008; JÜRGENS et al., 2010; AMEDE et al., 2023; OSEWE et al., 2023). Land-use conversion in combination with unsustainable agricultural practices has led to chemical, biological, and ecological degradation of approximately 25% of soils in SSA (LAL, 2015; AMELUNG et al., 2020; OKOLO et al., 2020; TEFERA et al., 2024). Among essential nutrients, nitrogen (N) is one of the most limiting yet important nutrients for crop growth (THILAKARATHNA et al., 2016; ABD-ALLA et al., 2023; CHANDA et al., 2023; LIU et al., 2025). Therefore, integrating legume crops, which can fix atmospheric N, is an important option to address N limitation in low-input systems (MACLAREN et al., 2022; RASCHE et al., 2023).

Namibia, located in the semi-arid region of SSA, exemplifies these challenges. Namibia is one of the driest countries in the region, and the majority of agricultural land in the north-eastern part consists of rainfed systems (AWALA et al., 2019; Siyambango et al., 2022; Shiimi & Uchezuba, 2025). Under rainfed conditions, sandy soils are highly vulnerable to degradation under current management practices (Hartemink & Huting, 2008; Thierfelder et al., 2015; De Blécourt et al., 2019; Soropa et al., 2019; Huang & Hartemink, 2020). Climate change further intensifies these problems (Critchley et al., 2023; Mutengwa et al., 2023; Osewe et al., 2023; Abshiba et al., 2025), with SSA representing a climate change hotspot (Engelbrecht et al., 2024). Climate change projections indicate temperature increases of 1.5-2.5 °C, rainfall reductions of 10-20% and increasing extreme weather events (Trisos et al., 2023; Engelbrecht et al., 2024), resulting in crop yield declines (Sultan et al., 2019; Ortiz-Bobea et al., 2021). Consequently, the area of arable agricultural land is expected to shrink, forcing smallholder farmers to convert natural ecosystems to agricultural land, thereby accelerating soil degradation (Amelung et al., 2020; Guja & Bedeke, 2024).

Agricultural land in northern Namibia is mainly cultivated by smallholder farmers, who have limited access to fertilisers and irrigation due to infrastructural and financial constraints (De Brauw & Bulte, 2021; Guja & Bedeke, 2024; Kirsten et al., 2024; Sithole & Olorunfemi, 2024). Unsustainable practices, such as continuous cultivation without nutrient inputs, intensive tillage, and removal of crop residues, have accelerated soil nutrient losses (Lal, 2015; Kopittke et al., 2019; Araya et al., 2024). This has resulted in self-reinforcing "low-input-low-output" production cycles, trapping many smallholder farmers in poverty and food insecurity (Pröpper et al., 2013; Lal, 2015; Taapopi et al., 2018; De Brauw & Bulte, 2021; Guja & Bedeke, 2024; Rötter et al., 2024).

As a consequence, declining soil fertility reduces microbial and enzymatic activity (Kopittke et al., 2019; Antwi-Agyei et al., 2025), which are crucial for nutrient cycling, OM stabilisation (Liu et al., 2024c; Jiang et al., 2025) and thus crop productivity (Chianu et al., 2011; Amelung et al., 2020; Okolo et al., 2020). Therefore, restoring or maintaining soil fertility with approaches like conservation agriculture (CA) and the integration of legume crops in cropping systems is essential to enhance soil microbial and enzymatic activity, resulting in agricultural resilience under the variable climatic conditions of Namibia (Lal, 2015; Amelung et al., 2020; Rötter et al., 2024; Abshiba et al., 2025).

Namibia, therefore, represents a case study for investigating sustainable soil management under semi-arid conditions, where rainfed smallholder farming systems dominate. Low-cost soil management strategies, such as legume-based cropping systems and CA, potentially enhance soil fertility and thus soil productivity, nutrient cycling, and C sequestration, thereby ensuring food security (Kopittke et al., 2019; Mutengwa et al., 2023; Araya et al., 2024; Wang et al., 2025b). However, long-term studies and knowledge on the combined effects of CA and legume cultivation on soil biological activity and C-N-dynamics, particularly in sandy soils of northern Namibia, remain limited. This dissertation, therefore, aims to investigate the potential of legume integration and CA to improve soil fertility and biogeochemical properties at different spatial and temporal scales, which is crucial for developing resilient and resource-efficient farming systems under semi-arid conditions.

1.1.2 Land management as a driver of soil fertility

In semi-arid smallholder systems in SSA, sustainable land management practices are crucial to improve soil fertility and crop productivity. Smallholder farms, less than 2 ha in size, account for more than 80% of all farms worldwide and more than 11% of these smallholder farms are located in SSA (BEKUNDA et al., 2010; LAL, 2015; GILLER et al., 2021; ARAYA et al., 2024; GUJA & BEDEKE, 2024). Agriculture in these systems is typically rainfed, characterised by low nutrient inputs and drought-resistant crops, such as pearl millet (*Pennisetum glaucum*), sorghum (*Sorghum bicolor*), maize (*Zea mays*) and cowpea (*Vigna unguiculata*) (PRÖPPER et al., 2013; AWALA et al., 2019; VANLAUWE et al., 2019; RASCHE et al., 2023; GUJA & BEDEKE, 2024; RÖTTER et al., 2024). In northern Namibia, about 98% of the population are smallholder farmers practising rainfed agriculture (PRÖPPER et al., 2013; AWALA et al., 2019).

To overcome soil fertility constraints, CA has been increasingly promoted as a climate-resilient and sustainable land management approach to improve soil fertility and maintain crop yields without further land conversion (KOPITTKE et al., 2019; MASETE et al., 2022; THIERFELDER & MHLANGA, 2022; ARAYA et al., 2024; CHAPUNGU & ZHANDA, 2025). According to the Food and Agriculture Organization of the United Nations (FAO) (2025), CA is based on three principles: minimum soil disturbance, permanent soil cover and crop rotation. In semi-arid regions like northern Namibia, CA can conserve water and regulate soil temperature (MUPANGWA et al., 2012; SINGH et al., 2021; MASETE et al., 2022), create favourable conditions for biological activity of beneficial soil microorganisms (MASETE et al., 2022; THIERFELDER & MHLANGA, 2022; ABSHIBA et al., 2025), improve soil organic matter (SOM) accumulation and suppress weeds and pathogens (PARWADA et al., 2022; THIERFELDER & MHLANGA, 2022; ARAYA et al., 2024). As one of the CA principles, minimum soil disturbance or tillage can improve physical, chemical and biological soil properties (SINGH et al., 2021; GUJA & BEDEKE, 2024; ABSHIBA et al., 2025). Soil OM is protected by conserved aggregates as a result of minimum tillage (PARWADA et al., 2022; ABSHIBA et al., 2025; Colunga et al., 2025), which binds OM and protects it against microbial decomposition, increasing resistance to erosion and enhancing C sequestration (Abdallah et al., 2021; Parwada et al., 2022; Colunga et al., 2025). Covering the soil with organic mulches further contributes to soil fertility by conserving water, maintaining favourable soil temperatures and providing organic substrate that improves microbial activity and thus soil nutrient cycling (El-Beltagi et al., 2022; Thierfelder & Mhlanga, 2022; Araya et al., 2024; Guja & Bedeke, 2024). Consequently, mulching has been reported to be the most important of the three CA principles in semi-arid smallholder systems to support short-term yield stability (Masete et al., 2022; Guja & Bedeke, 2024). While most organic mulch materials are scarce due to low net primary productivity, smallholder farmers have to decide whether to use them as mulch material for the soil, feed for livestock or construction material (Giller et al., 2009; Taapopi et al., 2018; Masete et al., 2022; Araya et al., 2024; Chapungu & Zhanda, 2025). Therefore, mulch generated through bush-clearing efforts to control shrub encroachment, particularly from *Acacia* sp., could provide a locally available mulch source in Namibia (Devine et al., 2017; Mupangwa et al., 2023; Zimmer et al., 2024). In addition, the CA practice of crop diversification brings many benefits compared to monocropping (Guja & Bedeke, 2024; Yang et al., 2026). Crop diversification or crop rotations commonly involve cereal crops that rotate with legume crops such as cowpea and soybean (Guja & Bedeke, 2024). Through crop diversification, the utilisation efficiency of limited nutrients might increase (Guja & Bedeke, 2024; Abshiba et al., 2025). In particular, the integration of legume crops into rotations further contributes to soil fertility as their ability to fix atmospheric N can help to reduce the dependence of high yields on high fertiliser inputs (Siddique et al., 2012; Hansen et al., 2017; Maclaren et al., 2022; Araya et al., 2024; Guja & Bedeke, 2024; Yang et al., 2026). In addition, SOC sequestration and storage are enhanced by root exudates and residue inputs from diversified cropping systems, which increase microbial activity and diversity, thereby improving nutrient cycling (Pandey et al., 2018; Pausch & Kuzyakov, 2018; Yang et al., 2026).

Although CA has been increasingly promoted as a sustainable agricultural approach, land-use adaptation to CA remains low in SSA, with less than 2% of adapted cropland under CA and only 8% of smallholder farmers practising all three CA principles (Araya et al., 2024; Shiimi & Uchezuba, 2025), mainly caused by limited resources of farmers and lack of knowledge (Mutengwa et al., 2023; Trisos et al., 2023). Only a few studies have investigated long-term effects on soil biogeochemical parameters in dryland systems (Corbeels et al., 2020; Mganga et al., 2024), particularly in Namibia (Thierfelder & Mhlanga, 2022), and the extent to which CA improves soil fertility remains to be evaluated. This knowledge gap limits understanding of CA’s potential to restore soil fertility and improve crop productivity under semi-arid conditions.

1.1.3 Legume crops in low-nutrient soils for soil fertility

Legume crops play an important role in maintaining soil fertility and enhancing SOM, particularly in low-nutrient soils in smallholder farming systems (GOGOI et al., 2018; KANTÉ et al., 2021; KEBEDE, 2021; AYALEW et al., 2024; SRIVASTAVA et al., 2025). They are widely used in intercropping or crop rotations in agricultural systems to supply N in the soil for following crops (THILAKARATHNA et al., 2016; KANTÉ et al., 2023), while also contributing to SOC through rhizodeposition (KANTÉ et al., 2021; VIRK et al., 2022). In semi-arid smallholder systems, where nutrient availability - especially N - is a major limitation (DE BLÉCOURT et al., 2019; SOROPA et al., 2019), the integration of legume crops into crop rotations becomes a key strategy for sustainable soil management (THILAKARATHNA et al., 2016; SAMBANGI et al., 2021; CHAPUNGU & ZHANDA, 2025). Legume crops can restore soil N through biological N fixation, a process in which atmospheric dinitrogen (N_2) is converted into plant-available forms through a mutualistic symbiosis with legume-nodulating rhizobia (DAKORA & PHILLIPS, 2002; SHARMA et al., 2017; GOGOI et al., 2018; EL SABAGH et al., 2020). This natural process accounts for up to 45% of global biological N fixation, reducing dependence on costly chemical N fertilisers (KEBEDE, 2021; ABD-ALLA et al., 2023; CHAPUNGU & ZHANDA, 2025). Through N fixation, soil N content can increase up to 40% (SRIVASTAVA et al., 2025), particularly in the rhizosphere, where N fixation in nodules and root exudation increase soil N and nutrient availability (SIDDIQUE et al., 2012; KEBEDE, 2021; SRIVASTAVA et al., 2025). The N fixation capacity of grain legumes was estimated to an amount of 113.4 kg N ha^{-1}, but depends on cultivar, soil, and rhizobial strain (DEAKER et al., 2004; KEBEDE, 2021; HERRIDGE et al., 2022).

Nodule formation is induced by root exudates acting as signals that attract rhizobia to root hairs, where they infect the root via a crack-entry mechanism, reproduce and form a nodule (DAKORA & PHILLIPS, 2002; EL SABAGH et al., 2020; KAZMIERCZAK et al., 2020; YEREMKO et al., 2025) (Figure 1). Rhizobia, gram-negative bacteria living within nodules, convert N_2 into ammonia for the plant, while rhizobia receive energy in the form of C compounds, such as carbohydrates or organic acids, from the plant (GEORGE et al., 2016; ABD-ALLA et al., 2023; CHANDA et al., 2023; LI & LI, 2023; THARANATH et al., 2024). Like other plant organs, the root nodule has a limited lifespan of approximately 10-12 weeks, with N_2 fixation rates declining after 3-5 weeks (KAZMIERCZAK et al., 2020). Once the lifespan is over, nodules enter a senescence process that is characterised by a decline in N_2 fixation and the death of rhizobia and plant cells (Kazmierczak et al., 2020; Berrabah et al., 2024). The senescence process can either be triggered by environmental conditions or can result from nodule ageing (Kazmierczak et al., 2020). At the plants' physiological maturity stage, rapid nodule decay occurs during nodule senescence (Kazmierczak et al., 2020), representing a N pathway in the soil (Kebede, 2021) that is considered more important than root exudation (Thilakarathna et al., 2016).

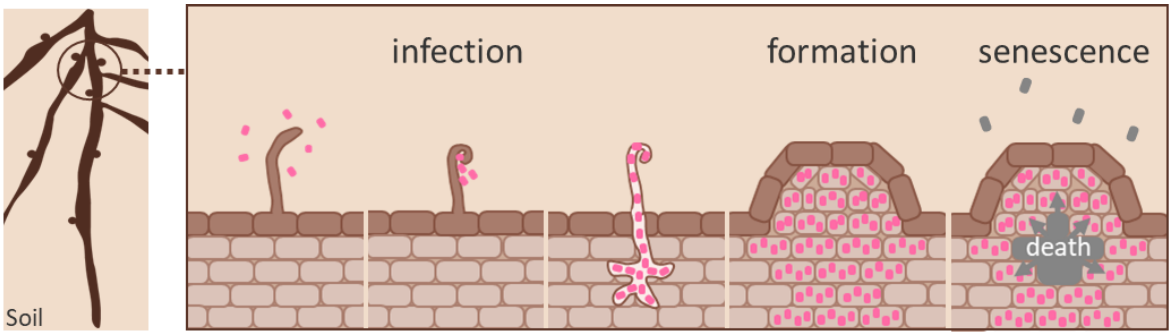

Figure 1: Life cycle of root nodules, beginning with rhizobial infection under low soil nitrogen content, followed by nodule formation and ending with nodule senescence. Pink colour represents rhizobia.

Beyond N inputs, legume crops contribute high-quality OM and root exudates that enhance nutrient availability for following crops (Siddique et al., 2012; Hansen et al., 2017; Kebede, 2021). These organic inputs can also stimulate soil microbial and enzymatic activity, contributing to SOC turnover and improved nutrient cycling (Bakshi & Varma, 2010; Thilakarathna et al., 2016; Srivastava et al., 2025).

One of the most important food grain legumes in SSA is cowpea (*Vigna unguiculata*) (Badiane et al., 2014; Vanlauwe et al., 2019; Horn & Shimelis, 2020; Rasche et al., 2023) (Figure 2). Cowpea is a key crop for food security due to its ability to grow under low water and high temperature conditions (Badiane et al., 2014; Ndiso et al., 2015; Carvalho et al., 2017). Cowpea grows well in a temperature range of 18-28°C (Badiane et al., 2014; Hayatu et al., 2014) under rain-fed conditions with a mean annual rainfall of 600 mm (Ndiso et al., 2015) and can fix up to 240 kg N ha^{-1} (Rasche et al., 2023).

Figure 2: a) Cowpea seed, b) germinated cowpea seeds, c) cowpeas at maturity stage, d) cowpea flower and e) root of harvested cowpea with nodules.

Rhizobial inoculation for enhanced N fixation

The potential of legume crops to fix N is closely linked to the rhizobial strain and the efficiency of rhizobia (ABD-ALLA et al., 2023; RASCHE et al., 2023). In regions of northeastern Namibia, cowpea shows a relatively low nodulation caused by drought and heat (GRÖNEMEYER et al., 2014). In such environments, inoculating legume crops with effective rhizobial strains provides a practical and low-cost approach to enhance nodulation and thus N fixation (DEAKER et al., 2004; LUPWAYI et al., 2011; FIGIEL et al., 2025). Through inoculation, the rhizosphere is rapidly colonised by effective rhizobia (DEAKER et al., 2004; LUPWAYI et al., 2011; FIGIEL et al., 2025; JEONG et al., 2025). Effective inoculation increases both N availability and plant growth, leading to higher crop yields (ABD-ALLA et al., 2023; RASCHE et al., 2023; CHEN et al., 2024; LAISHRAM et al., 2024). Thus, inoculation is environmentally friendly and reduces the use of costly chemical fertilisers (FUSTEC et al., 2010; CHIANU et al., 2011; ABD-ALLA et al., 2023; CHANDA et al., 2023; CHEN et al., 2024; LAISHRAM et al., 2024). However, successful inoculation depends on various abiotic and biotic factors, such as matching relationships between rhizobial strains and legume host, soil pH, soil texture and climatic conditions (GRÖNEMEYER et al., 2014; HORN & SHIMELIS, 2020; SHRIVASTAVA et al., 2021; ABD-ALLA et al., 2023).

Although legume crops are known to improve soil N status through biological N fixation in root nodules, the dynamics throughout plant growth, particularly during nodule senescence, remain poorly understood. Few studies exist on legume-soil interactions during plant growth (Fustec et al., 2010; Becker et al., 2024). How nodule senescence contributes to soil nutrient availability, and SOC remains poorly understood, particularly in low-nutrient soils.

1.1.4 Soil microbial and enzymatic activity to mediate nutrient cycles

Soil management practices and crops influence the habitat of soil microorganisms (Zuber & Villamil, 2016; Singh et al., 2021; Chen et al., 2024), but responses might differ depending on the investigated spatial scale. Soil microorganisms are highly sensitive indicators of land management, responding faster to changes in soil fertility than SOM (Zuber & Villamil, 2016; Adetunji et al., 2017; Malobane et al., 2020; Abshiba et al., 2025). During microbial decomposition of OM, nutrients are released (Pandey et al., 2018; Kaviya et al., 2019; Chen et al., 2024). Therefore, microbial parameters such as microbial biomass, microbial diversity and soil enzyme activities serve as key biological indicators of early changes in soil fertility (Lal, 2015; Adetunji et al., 2017; Malobane et al., 2020; Singh et al., 2021; Chen et al., 2024).

Soil microbial biomass is associated with soil processes like SOM decomposition and storage, nutrient cycling, as well as soil structure (Kaviya et al., 2019; Mganga et al., 2024). In low-nutrient soils, like in Namibia, microbial biomass represents the major reservoir of available nutrients and thus determines plant survival (Singh & Gupta, 2018). Changes in microbial biomass directly reflect management effects and shifts in soil resource availability, and soil enzyme activities reflect microbial functional responses and nutrient demand (Burns et al., 2013; Adetunji et al., 2017; Malobane et al., 2020).

Enzymes, released by roots and microorganisms, are key drivers of OM decomposition and nutrient cycling (Luo et al., 2017; Kuzyakov & Razavi, 2019; Liu et al., 2022; Wang et al., 2023a; Jiang et al., 2025). In the rhizosphere, soil enzymes regulate nutrient cycling and SOM mineralisation (Kuzyakov & Razavi, 2019; Liu et al., 2022; Liu et al., 2024a). Higher enzyme activities in the rhizosphere can be interpreted as a greater functional diversity of the microbial community (Gianfreda, 2015). Enzyme production is driven by the availability of nutrients and energy sources, which are strongly affected by the quality and quantity of root exudation and rhizodeposition during plant growth (Ge et al., 2017; Wei et al., 2019). Soil enzymes, such as β-glucosidase (BG), chitinase (NAG) and leucine-aminopeptidase (LAP), mediate the decomposition of SOM and are thus key enzymes for C- and N- cycling (Burns et al., 2013; Razavi et al., 2016b; LIU et al., 2024b). β-glucosidase is produced by plants and microorganisms, such as bacteria, and catalyses the hydrolysis of cellulose, releasing glucose, a primary energy source for heterotrophic microorganisms. It occupies a central role in the global C-cycle because it catalyses the final step in the breakdown of cellulose (LUO et al., 2017). Chitinase plays a crucial role in soil N acquisition by breaking down chitin and peptidoglycans, which serve as proxies for microbial turnover and N availability (LUO et al., 2017; CHEN et al., 2018; KUMAR et al., 2022). Leucine-aminopeptidase is used as a proxy for measuring protease activity, as it catalyses the cleavage of N-terminus amino acids from peptides and proteins (GREENFIELD et al., 2021). It plays a key role in plant rhizosphere processes, including the degradation of storage proteins, which are essential for plant development (LI et al., 2024a).

These enzyme-mediated processes mainly take place in the rhizosphere, which represents a hotspot area between roots and the surrounding soil (KUZYAKOV & BLAGODATSKAYA, 2015; RAZAVI et al., 2016b). In this area, rhizodeposition – the release of organic compounds from roots and nodules – occurs, which significantly contributes to soil C and N availability (KANTÉ et al., 2023; WANKHADE et al., 2025) and might not only influence the rhizosphere but also alter the bulk soil (LAL, 2015; PAUSCH & KUZYAKOV, 2018; KUZYAKOV & RAZAVI, 2019). Rhizodeposits, such as root exudates, are rich in labile C and generate a microbial hotspot for nutrient cycling, stimulating microbial activity (KUZYAKOV & BLAGODATSKAYA, 2015; RAZAVI et al., 2016b; KANTÉ et al., 2023). In addition, root death and nodule senescence further enhance the availability of C and N (KEBEDE, 2021; VIRK et al., 2022). This is particularly relevant in legume cultivation, which has accelerated root decomposition and nutrient release due to the low C:N ratio of legume plant tissues compared to other crops (BLESH, 2019; VIRK et al., 2022).

The interactions between plants and microbes mediated by enzymes vary during plant growth due to shifts in physiological processes and rhizodeposition (GE et al., 2017; MA et al., 2018a; SICZEK et al., 2018; WEI et al., 2019; KANTÉ et al., 2021). For legume crops, enzyme activities in rhizosphere and bulk soil have been shown to increase until the legume reproductive stage (MA et al., 2018a; SICZEK et al., 2018). Later, at maturity stage, increased root decomposition and nodule senescence lead to a temporary release of OM into the soil (KAZMIERCZAK et al., 2020; KANTÉ et al., 2023). This OM provides labile substrates for microbes, which in turn can stimulate enzyme production and thus influence nutrient cycling.

As enzyme activities directly reflect microbial access to C and N, enzymatic ratios can further indicate whether soil processes are limited by C or N (SINSABAUGH et al., 2008). In soils with low OM, like sandy soils in northern

Namibia, microorganisms may respond sensitively to management changes, like mulching under CA, or legume cultivation, which can alter enzyme activities (HUANG & HARTEMINK, 2020; TIAN et al., 2020; ISLAM et al., 2025). Therefore, enzyme activities represent a highly responsive and integrative indicator of biological soil quality.

These microbial-driven processes, such as enzyme production or SOM decomposition, are likely mediated by soil microbial communities (PANDEY et al., 2018; DANG & MORRISSEY, 2024). A microbial community with high diversity results in the release of different enzymes under varying soil conditions like pH, temperature, and water availability, which controls nutrient cycling and enhances ecosystem resilience (KAVIYA et al., 2019; CHEN et al., 2024; DANG & MORRISSEY, 2024). Thus, ecosystems with higher microbial diversity and biomass may have a greater capacity to sustain ecological processes (SINGH & GUPTA, 2018).

Despite their importance, detailed studies of microbial indicators, such as enzyme activities, in semi-arid agricultural systems like northern Namibia, remain scarce. Understanding these enzyme-mediated interactions between legume crops, microbial communities, and soil management is therefore essential to assess soil fertility improvement and develop sustainable management strategies for dryland agriculture.

1.2 Thesis objectives and outline

The aim of this dissertation was to find out how sustainable land management and legume cultivation can contribute to soil fertility improvement (SOC, N, microbial biomass) in northern Namibia at multiple spatial and temporal scales. On a field scale, different land-use types – CA, traditional agriculture (TA) and woodland (W) – and agricultural managements were selected to study effects of CA on smallholder farms and two Namibian agricultural regions were selected as exemplary regions to study effects of CA, with a particular focus on legume cultivation, under experimental conditions. Further, in a small-scale greenhouse experiment under laboratory conditions, effects of CA (mulching and legume cultivation) and inoculation under drought conditions were analysed using low-fertility soils from the exemplary regions. At the smallest spatial scale, legume effects on rhizosphere C- and N-dynamics were investigated. Understanding soil improvement across spatial scales and under different conditions, using the described sustainable and low-cost tools, is essential to ensure food security for smallholder farmers and to improve agricultural management. Apart from management practices like mulching, a particular focus was on investigating how legume cultivation contributes to SOM accumulation. To improve the understanding of these effects in low-fertility soils under semi-arid conditions, this dissertation is structured around three studies (Figure 3):

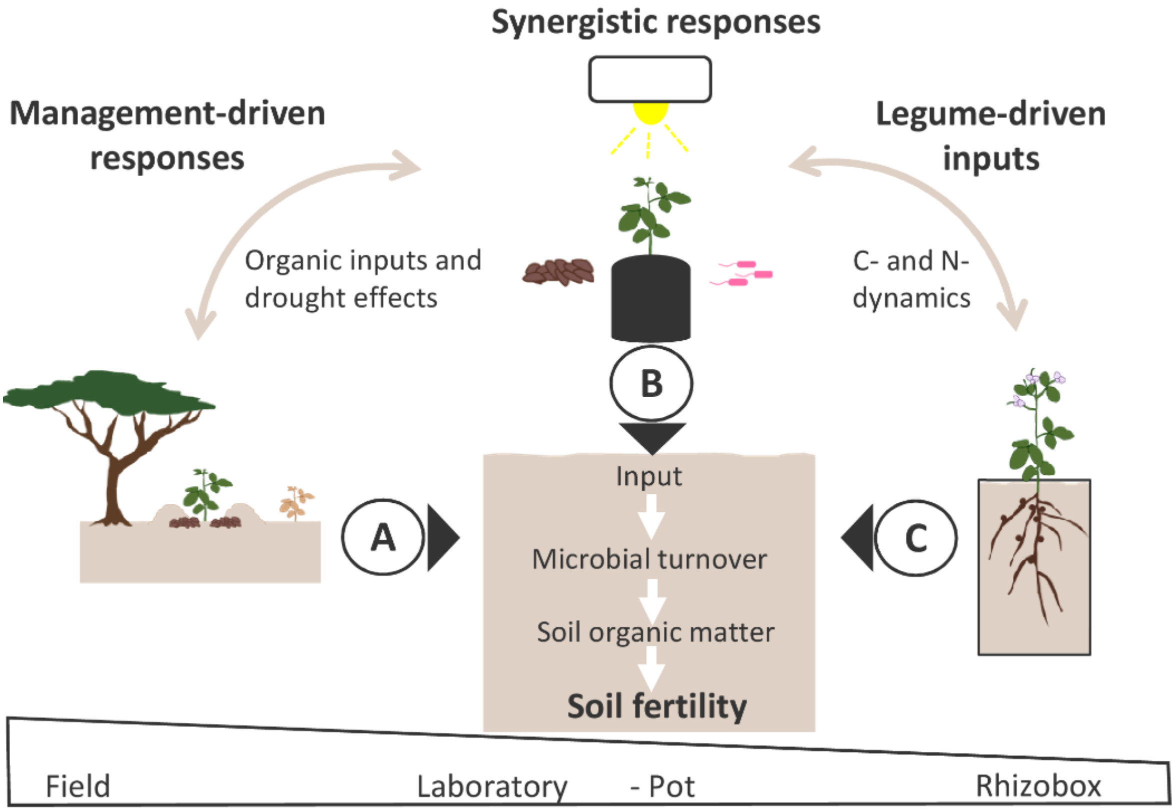

Figure 3: Scheme of the different studies related to this dissertation (Studies A, B and C) conducted at different experimental scales under field and controlled laboratory conditions. Study A was conducted in the field, Study B was conducted as a pot experiment in a greenhouse and Study C was conducted under laboratory conditions in rhizoboxes. Study A focuses on soil chemical and microbial responses to different land-use types and management practices. Study B investigates management-driven inputs, such as mulching and legume cultivation combined with inoculation, and their effects on soil biogeochemical properties under different soil water availabilities. Study C focuses only on legume-driven inputs and their effects on carbon and nitrogen dynamics. Across all studies, inputs influence microbial turnover and soil organic matter dynamics, affecting soil fertility with potential synergistic responses between management- and legume-driven effects, including drought conditions. C= carbon, N= nitrogen.

Study A

The objective of Study A was to investigate effects of land management adaptation from CA to TA on (I) C and N content in the soil, (II) the potential crop yield based on soil fertility, and (III) microbial community in a field study.

Study B

The objective of Study B was to investigate whether (I) mulch application and different mulch types increase SOC as well as TN content and enhance enzyme activities, (II) rhizobial inoculation increases N availability, and (III) the combination of mulch and inoculant further amplifies these responses (IV) while mulch buffers negative effects of low water availability.

Study C

The objective of Study C was to investigate legume-soil interactions by examining (I) rhizosphere and bulk soil enzyme activities over the course of plant growth, (II) particularly after nodule senescence, and (III) soil type effects on the spatio-temporal enzyme response.

Embedment within the SUSTAIN project

This doctoral project is embedded in the SUSTAIN "Sustainable Food Security and Woodland Utilization for Drought-Prone Communal Areas under Climate Change in SADC Countries" project that belongs to the collaborative initiative SASSCAL 2.0. The overarching aim of this initiative is to explore local and regional climate adaptation in SSA through transregional and interdisciplinary collaboration. The project is funded by the Federal Ministry of Research, Technology and Space (01LG2051B). The shared research objective of the SUSTAIN project is to address existing knowledge gaps to strengthen local resilience and advance climate adaptation in SSA, with a focus on areas that concern food security, water security, biodiversity conservation, sustainable forestry and forest use, as well as climate services. The SUSTAIN project involves an interdisciplinary group of researchers with diverse scientific backgrounds, including soil science, microbiology, and socio-economists. Additional support was received through CLICCS (Climate, Climatic Change and Society), funded by the Deutsche Forschungsgemeinschaft (DFG) under Germany's excellence strategy, EXC 2037 CLICCS (390683824), as part of the University of Hamburg.

2 Material and methods

2.1 Study area: Namibia

The Kavango and Omusati regions, located in Northern Namibia, were selected as exemplary regions with low-fertility soils for all studies of this dissertation (Figure 4). Both regions extend along the Angolan border in the north, with the Kavango region reaching eastwards to Mururani and the Omusati region southwards to the Etosha Basin. This area in northern Namibia covers a gradient of declining water availability from the Okavango floodplain to drier inland plateaus. Both regions are characterised by smallholder farming under water-limited climatic conditions. The regions have a semi-arid climate (PRĂVĂLIE, 2016; RÖTTER et al., 2024), with mean annual temperatures of 22.4 °C and average annual rainfall of 450 - 550 mm, occurring mainly between November and March (AWALA et al., 2019). Rainfall determines the length of the growing period, which ranges from 60 to 119 days in the Kavango and Omusati regions (RÖTTER et al., 2024).

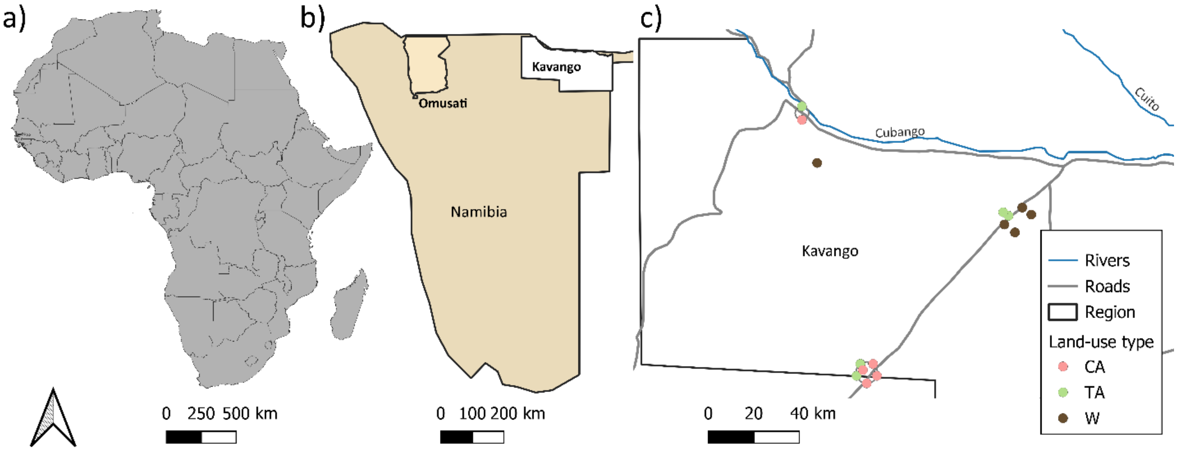

Figure 4: a) Location of Namibia (brown filling) in Africa (grey filling) and b) location of the major agricultural regions Kavango (white) and Omusati (light brown) in northern Namibia, c) sites within the Kavango region under conservation agriculture (CA), traditional agriculture (TA) and woodland (W) with five sites per land-use type (created with QGIS; data sources: Natural Earth).

Studies B and C are based on topsoil (0-10 cm) samples from experimental field sites established within the framework of the SUSTAIN project in collaboration with local universities (Figure 4b). The Mashare fields were established in cooperation with the MADI (Mashare Agriculture Development Institute). The study site is located in the north-eastern Kavango region at an altitude of 1068 m a.s.l. (S 17° 53′ 27′′; E 20° 10′ 17′′), embedded in an old floodplain of the Okavango River (GRÖNGRÖFT et al., 2013). The soil is a loamy sand and was classified as Luvisol (BECKER et al., 2024), developed on translocated sands and dunes of the Kalahari Basin with interspersed clay and silt layers from fluvial deposits. The Ogongo study site is located in the Omusati region of central-northern Namibia, at an altitude of 1108 m a.s.l. (S 17° 41′ 08′′; E 15° 18′ 01′′) on the UNAM (University of Namibia) Ogongo campus. The area is covered by Kalahari sands on slightly elevated terraces that are partly flooded by watercourses (so-called "oshanas") that arise in the Angolan highlands during austral summer (JÜRGENS et al., 2010). The soil is sandy and was classified as Eutric Sideralic Arenosol (BECKER et al., 2024). These site-specific conditions have resulted in the formation of distinct soil and vegetation types, ranging from sandy Arenosols on uplands to Luvisols in low-lying depressions (HARTEMINK & HUTING, 2008), with Arenosols being the most common soil type (RÖTTER et al., 2024).

Soil samples (0-10 and 10-20 cm) for Study A were collected in the Kavango Region (Figure 4c), where most of the agricultural land is managed by smallholder farmers practising traditional rainfed agriculture. Common crops include pearl millet (*Pennisetum glaucum*), maize (*Zea mays*), and legume crops such as cowpea (*Vigna unguiculata*), which are mainly cultivated for household consumption (OLDELAND et al., 2013; AWALA et al., 2019; HORN & SHIMELIS, 2020; SIYAMBANGO et al., 2022). As described, soils are typically sandy with low OM and nutrient contents, which limit productivity and make sustainable soil management practices crucial for food production (PRÖPPER et al., 2013; DE BLÉCOURT et al., 2019; THIERFELDER & MHLANGA, 2022).

2.2 Study design

The study design combined field and laboratory experiments to assess how land management practices, environmental conditions, legume crop integration, and rhizobial inoculation affect biogeochemical soil properties, such as soil enzyme activity and crop yield in low-nutrient soils of northern Namibia. Field studies were conducted on experimental field sites (Appendix 1) and smallholder farms under different land-use types, while laboratory experiments aimed to simulate semi-arid farming conditions.

The research studies are designed as follows:

Different land use types in the Kavango region (Study A)

For field Study A, three land-use types were selected within the Kavango region: CA, TA and woodland (W), each with five replicates (Figure 5). The selection of the study sites was carried out in cooperation with the GIZ (deutsche Gesellschaft für Internationale Zusammenarbeit).

Sites under CA were managed by *leader farmers* trained by the GIZ according to the three principles of the Fao (2025): minimum soil disturbance, permanent soil cover and crop rotation. Minimum soil disturbance was achieved with the ripper-furrowing method, in which furrows approximately 30 cm deep are created to loosen the soil. The CA fields have been managed under these practices for at least six years, primarily through crop rotations with cowpea and pearl millet.

Under TA, farmers do not irrigate, apply fertilisers or use herbicides and pesticides (Grönemeyer et al., 2014). Soils are usually prepared with a plougher, without soil cover and monocropped (Uchezuba & Mbai, 2020; Siyambango et al., 2022).

The W reference sites were located within the community forest in the Kavango region and represented near-natural reference areas. Woodland sites were characterised by shrubs, grasses and woody vegetation. The sites showed no visible signs of grazing or agricultural disturbance and were therefore considered as near-natural reference sites.

Traditional Agriculture Conservation Agriculture Woodland

Figure 5: Different land-use types related to Study A in the Kavango region from left to right: traditional agriculture, conservation agriculture and woodland.

Experimental field sites

Two experimental field sites were established in the Kavango and Omusati regions to represent different soil and geological conditions to test land management practices, legume crop varieties, inoculation effects and mulch types (Appendix 2, Appendix 3, Appendix 4). Both sites included the two management systems from Study A, CA and TA (Figure 6). Within TA, plots included both inoculated and uninoculated treatments. Within CA, additional treatments were implemented, including mulch application with a mixture of wood chips and wheat straw, no mulch application, and inoculated and uninoculated soils. Both management practices were tested with two different cowpea varieties (*Silwana* and *Nakare*). The field sites thus allowed the systematic assessment of management interactions under representative environmental conditions of northern Namibia.

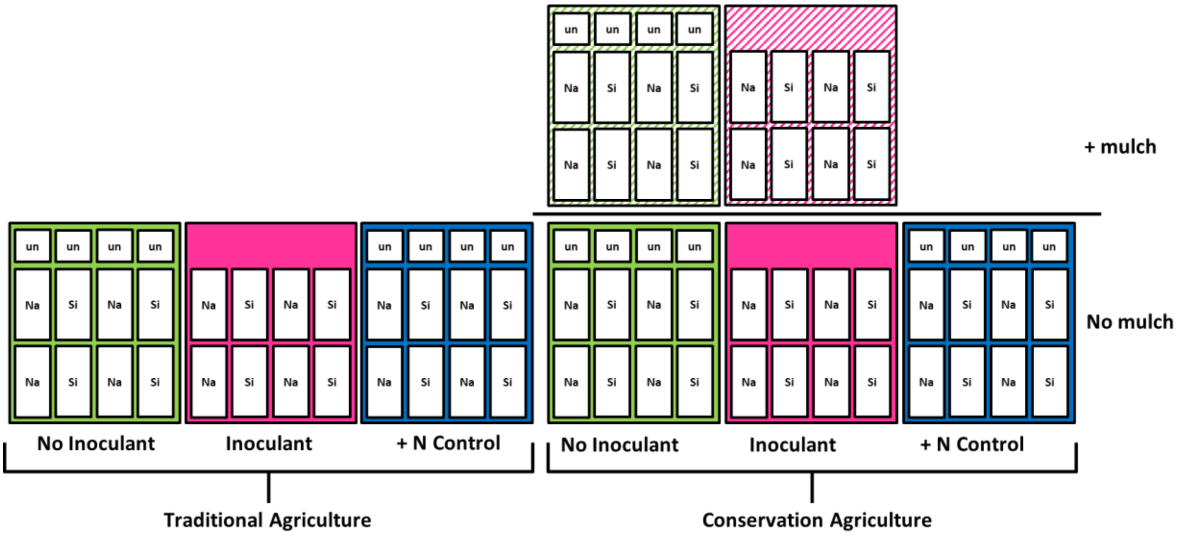

Figure 6: Simplified study design of the experimental field sites set up in cooperation with the University of Namibia in Ogongo and the Namibian University of Science and Technology in Mashare. Na= Nakare, Si= Silwana, un= unplanted plots, N= nitrogen.

Greenhouse pot experiment (Study B)

To identify effects of CA and the interaction with legume inoculation on soil biogeochemical properties, Study B was conducted as a greenhouse experiment (Figure 7). The experiment was conducted in a greenhouse using pots (12 cm in height, 10 cm in diameter) filled with sandy soil from the Omusati region. The experiment followed a factorial design combining two water levels: water optimum (45% of max. water holding capacity (WHC)) and water stress (20% of max. WHC) with four mulch treatments: with no mulch (to represent

TA), with *Acacia* sp. wood chips (WC), with wheat straw (WS), and with a 1:1 combination of both (WC + WS). Mulch treatments were designed to simulate CA practices, with mulch applied to fully cover the soil surface. In addition, directly after sowing, pots were either inoculated with 1 ml *Bradyrhizobium* sp. strain 26-nodO, with Modified Arabinose Gluconate medium, in liquid culture (referred to as inoculated) or left uninoculated but treated with 1 ml of sterile liquid Modified Arabinose Gluconate medium (referred to as uninoculated). The rhizobial strain had been isolated from nodules of the cowpea variety Bira grown at the UNAM campus in Ogongo (A. Sarkar and B. Reinhold-Hurek, unpublished results). Unplanted pots, per water level and treatment, served as controls. Each treatment included four replicates, and treatment-specific water content was maintained by weighing and watering each pot every four to five days. Cowpea (variety *Lutembwe*) seedlings were planted and grown until more than 50% of the plants under water optimum conditions had developed pods. Experimental conditions were controlled and designed to simulate semi-arid growing conditions, with day and night temperatures of 30 °C and 15 °C, a relative humidity of 50%, and a natural daylight cycle of 12 h (6:00-18:00), with natural daylight and additional illumination via plant lights when necessary.

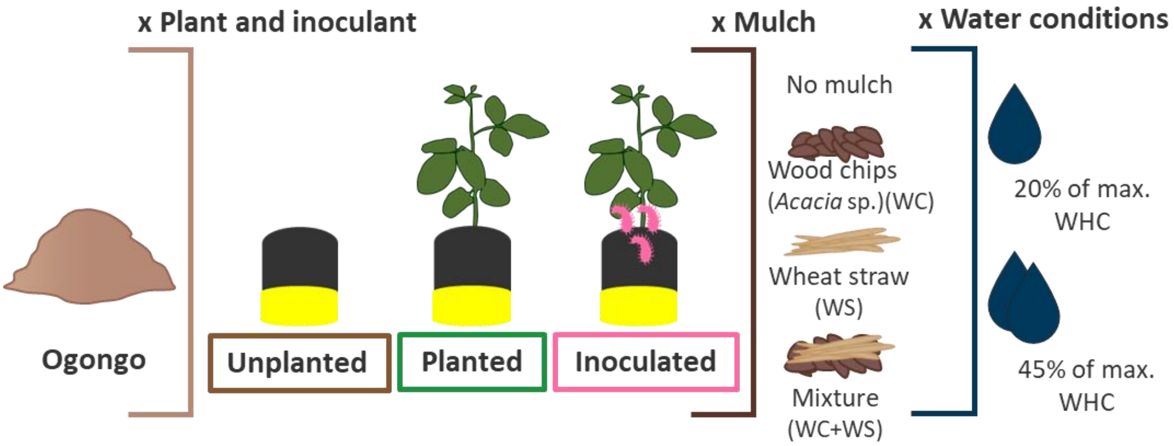

Figure 7: Simplified experimental setup of the greenhouse pot experiment from Study B to investigate mulch, inoculation and their interaction effects under different soil water availabilities on soil chemical and microbial properties. Unplanted= pots without cowpea, planted= pots planted with cowpea, inoculated= pots with inoculated cowpea, max. WHC = maximum water holding capacity.

Rhizobox experiment (Study C)

To investigate spatial and temporal responses in enzyme activity to legume cultivation, a laboratory experiment was designed to simulate a full plant growth period under controlled conditions (Figure 8). Cowpea (*Vigna unguiculata*) was grown in rhizoboxes (28 x 28 x 1 cm) and treated to a near-constant water availability of 45% max. WHC by weighing and watering each box every 48 hours. Six replicates of each soil were grown until maturity stage (or until nodule senescence appeared). The rhizoboxes were inclined at 60 ° to ensure root growth toward the transparent acrylic glass. Plants were grown at 22 °C and relative humidity of 50% under artificial illumination (six 200 μmol s^{-1} LED grow lights) with a photoperiod of 12 h per day.

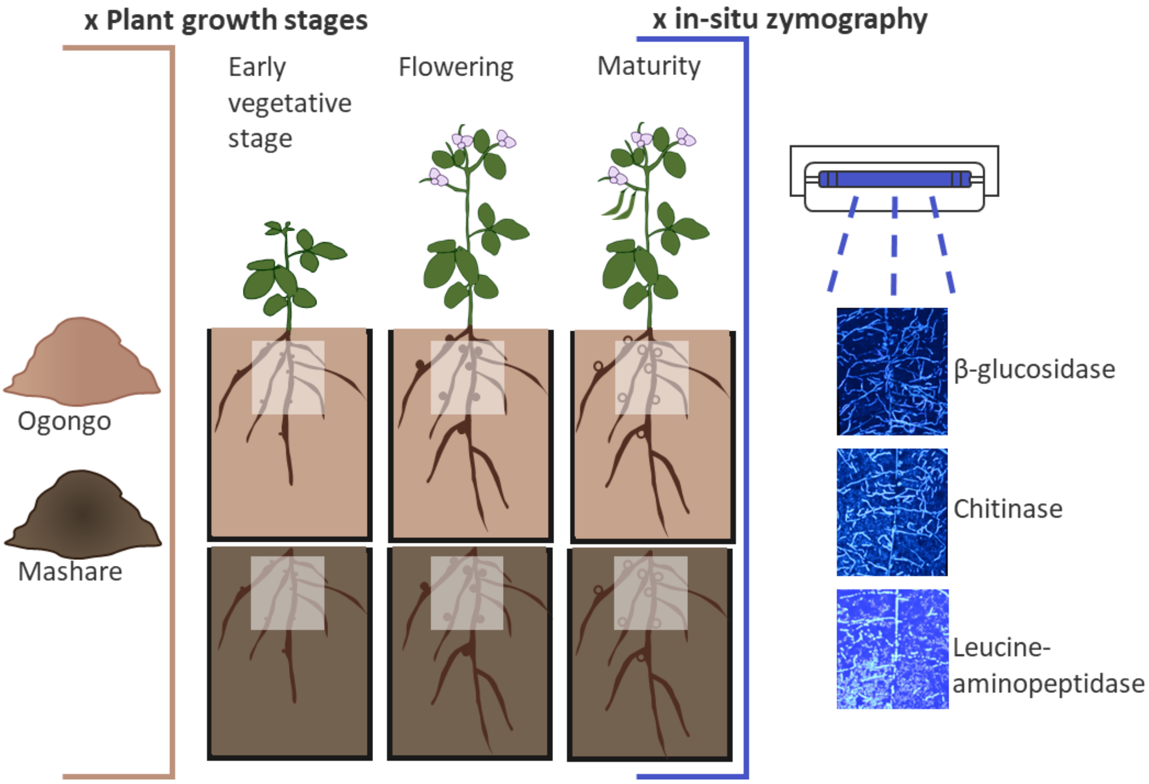

Figure 8: Schematic experimental setup of the rhizobox experiment from Study C to investigate spatial and temporal responses in enzyme activity to legume cultivation with two different soils from Mashare (loamy sand) and Ogongo (sand).

2.3 General soil characterisation

2.3.1 Physico-chemical soil characterisation

Soil (0-10 cm, 10-20 cm) samples were collected from different land-use types in the Kavango region for Study A. In addition, topsoil (0-10 cm) samples were collected from the experimental field sites in the Kavango and Omusati regions for Studies B and C. All samples were analysed for the general physical and chemical soil characterisation. Additionally, disturbed soil cores for bulk density analysis were derived from 0-10 cm and 10-20 cm for Study A. Physico-chemical soil properties were characterised by SOC, TN, mineral N (N_{min}), soil texture, soil pH, and electrical conductivity. In Addition, exchangeable cations (calcium, potassium, magnesium, sodium) and plant available phosphorus (P) were analysed for Study A. For a detailed description, see Studies A, B and C.

Soil bulk density was measured on samples taken at the plot centre using 100 cm^3 cylinders at two depths for Study A. The soil core samples were oven-dried at 105 °C. The weight of the oven-dried samples was divided by the core volume to calculate the bulk density. Soil texture was analysed using the sieving and sedimentation method based on the Köhn-pipette fractionation for mineral soils. A sieve tower (Retsch GmbH, Haan, Germany) was used to analyse the coarse fractions (2.0-0.063 mm), while a Sedimat 4-12 (Umwelt-Geräte-Technik GmbH, Müncheberg, Germany) was used to examine the fine fractions (<0.063 mm).

2.3.2 Soil fertility assessment

To quantitatively assess soil fertility under different land-use types, the Quantitative Evaluation of the Fertility of Tropical Soils (QUEFTS) model was applied (JANSSEN et al., 1990; SATTARI et al., 2014). The model estimates potential crop yields based on empirical and theoretical relationships between actual soil properties, soil nutrient supply and plant uptake, with N, P and potassium as the only growth-limiting nutrients. For a detailed description, see Study A.

2.4 Microbial soil parameters

2.4.1 Microbial biomass and basal respiration

Microbial biomass was determined using the chloroform fumigation extraction method, adapted from VANCE et al. (1987). Fresh soil samples were watered and extracted with 0.05 M K_2SO_4. Another fresh soil sample 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. The amount of microbial biomass C (C_{mic}) and microbial biomass N (N_{mic}) was determined by the difference between C and N in fumigated and non-fumigated soils. For a detailed description, see Studies A and B.

To estimate basal respiration (R_B), fresh soil samples were pre-incubated for one week. To measure R_B, the soils were placed in plastic tubes to capture carbon dioxide (CO_2) production using the flow-through method (Heinemeyer device, MarCo Analytik, Hildesheim) over 24 h with an infra-red gas analyser (CIRAS 3-DC). To evaluate the raw data, the SIR-SBA 6.03 program was used. For a detailed description, see Study A.

The metabolic quotient (qCO_2) was calculated by dividing basal respiration (CO_2 efflux of R_B) by C_{mic}.

2.4.2 Microbial community analysis

Microbial community composition was analysed using next-generation amplicon sequencing. Raw sequencing data were processed following amplicon sequence processing, including quality filtering, amplicon sequence variant inference and taxonomic assignment based on CALLAHAN et al. (2016) and MURALI et al. (2018). Community diversity and composition were analysed using α-diversity indices and multivariate ordination methods based on Bray-Curtis dissimilarities. For a detailed description, see Study B.

2.4.3 Enzyme assays

Activities of the extracellular enzymes were determined using an enzyme assay with synthetic fluorogenic substrates either with 4-methylumbelliferon (MUF) or 7-amino-4-methylcoumarin (AMC), followed by the protocol of MARX et al. (2001). The fluorogenic substrate used to detect BG was MUF-β-gluco-pyranoside, for NAG it was MUF-N-acetyl-ß-D-glucosaminide, and for LAP it was L-leucine-AMC-hydrochloride, respectively. All substrates and chemicals were purchased from Sigma-Aldrich (Darmstadt, Germany). The soil enzyme activities were quantified by calibration. Extracellular enzyme stoichiometry 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. (2024b) and YANG et al. (2020). 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 C:N ratio of 1:1 would represent an equilibrated system, values > 1 indicate C limitation. For a detailed description see Study B.

2.4.4 Soil zymography and imaging processing

Soil zymography was conducted to visualise the distribution of extracellular enzymes on the soil surface, around roots and nodules over time. We followed the protocol proposed by RAZAVI et al. (2016b). This involved visualisation of enzyme activities using membranes saturated with 4-MUF-substrates and 7-amino-4-AMC-substrates. These substrates become fluorescent when enzymatically hydrolysed by the respective enzyme. 4-methylumbelliferyl-β -glucopyranoside was used as substrate to detect BG activity; 4-methylumbelliferyl-N -acetyl-β-D-glucosaminide was used as substrate to detect NAG activity; and L-Leucine-7 -amido-4-methylcoumarin hydrochloride for LAP activity. InvitrogenTM Nylon membranes (Thermo Fisher, Germany) were saturated with the substrates for each enzyme. Membranes were placed under ultraviolet illumination in a light-proof chamber (Dark Hood DH-50, biostep GmbH). Constant conditions were maintained for all samples. Image processing and analysis were performed using the open-source software ImageJ (Java) and MATLAB (R2024a). For a detailed description, see Study C.

2.5 Statistical analyses

Differences in target variables were evaluated by analysis of variance with TukeyHSD post-hoc comparison or linear mixed-effect model in case of nested or repeated-measurement designs. Statistical significance was determined at p-values below 0.05, while p-values between 0.05 and 0.10 were considered as a tendency towards significance. All statistical analyses were performed in R 4.5.0 (R Core Team, 2025). For a detailed description, see Studies A, B, and C.

3 Key findings

Table 1: Overview table of the conducted studies (A, B and C), respective objectives and key findings related to this dissertation. Land-use types for Study A included conservation agriculture (CA), traditional agriculture (TA) and woodland (W). SOC= soil organic carbon, TN= total nitrogen, QUEFTS= Quantitative Evaluation of the Fertility of Tropical Soils, OM= organic matter, N= nitrogen, C= carbon.

<table>
<tr><th colspan="2">Field study: A</th></tr>
<tr><td colspan="2">Study A: <i>Soil carbon, nitrogen and microbial responses under different land-use types in Northern Namibia</i></td></tr>
<tr><td>Objectives</td><td>Key findings</td></tr>
<tr><td>• To assess the effect of CA on soil nutrients, microbial parameters and crop yield in the Kavango region
• To quantify SOC stocks under different land-use types
• To quantify the potential crop yield with the QUEFTS model
• To assess microbial efficiency by analysing microbial communities</td><td>• Time after land-use adaptation and site-specific conditions appeared to be key factors, since soil chemical properties, like SOC and TN, did not significantly increase under CA compared to TA
• Agricultural land use (TA and CA) decreased SOC stocks compared to W
• Potential crop yields tended to be higher under CA than TA
• α-diversity of microbial communities was significantly higher in CA compared to W</td></tr>
<tr><td colspan="2">Laboratory studies: B and C</td></tr>
<tr><td colspan="2">Study B: <i>Mulching, rhizobial inoculation, and water supply shape soil enzyme activities and nutrient dynamics in a Namibian sandy soil under cowpea cultivation</i></td></tr>
<tr><td>Objectives</td><td>Key findings</td></tr>
<tr><td>• To identify the effect of different mulch types, inoculation and their interaction under different soil water conditions on soil chemical and microbial properties</td><td>• SOC, TN and microbial biomass were more affected by cowpea cultivation and inoculation than by short-term mulching and mulch type
• Effects of mulch on soil chemical properties and enzyme activities were water-dependentInoculation improved N availability and led to a shift from N towards C limitation under optimum water conditions</td></tr>
</table>

Study C: *Spatio-temporal distribution of enzyme activities in cowpea rhizosphere – the role of plant growth stages and nodule senescence*	
Objectives	**Key findings**
• To determine the spatial and temporal dynamics of legume cultivation on C- and N-related enzyme activities in bulk and rhizosphere soil • To evaluate the effect of plant growth and nodule senescence on enzyme activities in two soils with different fertility • To gain detailed insights into soil improvement under legume cultivation	• Cowpea root exudation maintained stable enzyme activities at root and nodule surfaces and in rhizospheres during cowpea growth in both soils • Senescing nodules (occurred only in the sandy soil) represented a labile OM input, thereby increasing enzyme activities • Substrate availability through cowpea cultivation regulated bulk soil enzyme activities during plant growth in both soils • Soil type and properties affected enzyme activities, with higher bulk soil enzyme activity in loamy sand than in sandy soil • Cowpea cultivation outbalanced initial differences of SOC and TN between soil types, improving specifically soil fertility in the sandy soil

3.1 Soil management as a driver of soil fertility

Land-use type effects on soil chemical properties

At the field scale, SOC stocks and TN content were strongly influenced by land-use type, with W having the highest SOC stocks (0-20 cm) and TN content compared to agricultural sites (Figure 9). Agricultural sites (CA and TA) had comparable SOC stocks and TN contents. Contrary to findings in literature (THIERFELDER & MHLANGA, 2022; OLABANJI & CHITAKIRA, 2025), SOC stocks and TN contents under CA compared with TA did not increase. Higher SOC stocks in W compared to agricultural sites likely reflect SOC losses through land-use change. Similar patterns of SOC and TN were reported by DE BLÉCOURT et al. (2019) in Namibian W and agricultural sites. They similarly found no clear differences among agricultural sites in SOC and TN stocks, but increasing TN (0-10 cm) and SOC losses (10-20 cm) linked to age of agricultural sites. Apart from age, such losses can also be attributed to reduced nutrient inputs, lower root turnover and greater soil disturbance on agricultural sites (DE BLÉCOURT et al., 2019; TAYLOR et al., 2021; ARAYA et al., 2024; ABSHIBA et al., 2025). Based on defined thresholds of 2% SOC and 0.2% TN for productive soils (LANDON, 2014; OLDFIELD et al., 2019), all sites, including W, exhibited a low-nutrient status below these thresholds. Such low SOC contents, especially in SSA, were associated with depletion caused by conventional and intensive farming (RÖTTER et al., 2024). This low soil fertility status was supported by QUEFTS-based estimates, which identified N and P as the most yield-limiting nutrients in agricultural soils, whereas only P limited biomass production in W (Figure 9). These findings demonstrate the low fertility of these sandy soils and likely explain the limited response of soils to management adaptations observed at field scale.

SOC and TN responses to mulching

Mulching had limited effects on soil chemical properties, since SOC did not accumulate in the short-term - after one crop cycle (Study B), nor after six years (Study A). Instead, water availability emerged as the driver of SOC dynamics across studies (Studies A and B). Under greenhouse conditions, potential mulch-induced increases in SOC became apparent only under optimum water conditions, as reflected by higher SOC contents in mulched soils compared to water stress conditions (Study B; Figure 9). Such SOC increases were attributed to better plant growth that results in higher root exudation and accelerates microbial OC turnover, leading to C stabilisation (LIANG, 2020; DENG et al., 2021; QU et al., 2023; THAPA et al., 2023; SRIVASTAVA et al., 2025; WANG et al., 2025b). A review conducted by THAPA et al. (2023) suggests that sufficient water availability in sandy soils is essential to support plant growth, which has significant effects on SOC. In line with this, the limited effects of mulch on soil chemical properties observed in our study can be attributed to the short experiment duration (84 days) and, unlike other studies (MASETE et al., 2022; ROSSI et al., 2024), insufficient organic inputs to alter bulk soil properties (SAPKOTA et al., 2015; SUN et al., 2021; NOOR UL et al., 2022; ORTEGA et al., 2023; ROSSI et al., 2024). Moreover, under laboratory conditions, mulch decomposition is slow in the absence of decomposers such as termites (GILLER et al., 2009; NJOROGE et al., 2022; XU et al., 2024) (Study B). Whereas under field conditions, SOC accumulation might be limited by rapid decomposition and low mulch quantities applied by farmers, probably explaining the absence of detectable differences between CA and TA (Study A). Taking smallholder farmers' resources into account, continuous implementation of CA remains challenging. Especially type and amount of applied mulch likely influence nutrient dynamics and SOC accumulation (MASCIANDARO et al., 2004; EL-BELTAGI et al., 2022; MASETE et al., 2022; COLUNGA et al., 2025). Applying mulch amounts around 5 t ha^{-1} were reported to increase SOC (MASETE et al., 2022) compared with amounts less than 2 t ha^{-1} (GILLER et al., 2009). However, even small mulch application rates (2 t ha^{-1}) can be problematic for smallholder farmers (GILLER et al., 2009) as the availability of mulch materials (e.g. wheat straw, crop residues) is limited due to low biomass production in semi-arid regions. Smallholder farmers have to decide whether to apply mulch to fields, feed livestock or use it as construction material (GILLER et al., 2009; TAAPOPI et al., 2018; ARAYA et al., 2024). Additional risks like wind deportation of lightweight mulches (wheat straw) (APPIAH et al., 2023; AMIRI GHANAT SAMAN et al., 2025) or loss of mulch due to soil fauna, such as termites (GILLER et al., 2009; ARAYA et al., 2024), might reduce overall mulch effects under field conditions. Together, these constraints limit the feasibility of mulching as a CA principle under smallholder farming conditions (GILLER et al., 2009; MASETE et al., 2022; PARWADA et al., 2022; ARAYA et al., 2024; CHAPUNGU & ZHANDA, 2025). Another possible explanation for the lack of a clear difference in soil chemical properties between CA and TA might be potential local variation in adaptation, including slash-and-burn, intercropping with legumes or legume inoculation (Study A). Although we selected sites that fulfil criteria for TA, smallholder farms are heterogeneous and adaptation strategies that we cannot completely exclude might explain similar SOC and nutrient levels in CA and TA (VANLAUWE et al., 2019). Such adaptation strategies, like intercropping with legumes, were documented for smallholder livestock farmers (SPEAR & CHAPPEL, 2018; SIRIRIKA et al., 2025).

The similar effects of mulch types on soil properties imply that mulch quality plays a minor role for short-term SOM turnover in sandy soils of the Omusati region (Study B), although type and applied mulch influenced nutrient dynamics and SOC accumulation in other studies (MASCIANDARO et al., 2004; EL-BELTAGI et al., 2022; MASETE et al., 2022; COLUNGA et al., 2025). Nonetheless, *Acacia* sp. wood chips might provide better protection against wind erosion, rainsplash and surface runoff caused by their greater weight (APPIAH et al., 2023; AMIRI GHANAT SAMAN et al., 2025) compared to wheat straw mulch. Thus, wood chips from acacia bushes remain a practicable and sustainable mulch alternative to protect against soil erosion, even if short-term improvements of soil chemical properties are limited. However, the combination of mulch with inoculant resulted in increased TN independent of water availability, suggesting synergistic N benefits in low-fertility soils (Study B).

Soil management effects on plant biomass production

Plant biomass production was primarily limited by water availability and soil fertility across both field and laboratory studies (Study A and B; Figure 9). To compare soil fertility among different land-use types, potential crop yields were calculated for CA, TA and W. Predicted crop yields were highest in W, representing near-natural conditions, followed by CA and TA (**Figure 9**). Although differences in potential crop yield between CA and TA were not statistically significant, a trend towards higher yields under CA was observed. Yield increases under CA were also found in previous studies and likely explained by effects of mulching and crop rotations, but also linked to increased soil fertility under CA in SSA (Corbeels et al., 2020; Phiri et al., 2025). However, compared to yields reported for similar land-use types in Namibia (Pröpper et al., 2015; De Blécourt et al., 2019), predicted yields in our study were lower. This generally low yield potential likely reflects site-specific limitations (Huang & Hartemink, 2020), particularly the sandy soil texture, which limited biomass production even under near-natural conditions in W. At agricultural sites, we assumed that our low predicted yields indicate pronounced nutrient depletion resulting from land-use conversion from W to cropland, combined with insufficient nutrient inputs to balance losses as reported in other studies (De Blécourt et al., 2019; Huang & Hartemink, 2020; Tefera et al., 2024). As a result, soil fertility has declined to levels that cannot support higher crop yields in smallholder farming systems in Namibia, indicating that current agricultural management is unsustainable. Results from Study B might partly explain observations from field experiments. Drought conditions increased the root:shoot$_{\text{ratio}}$, indicating a shift in biomass allocation (Eziz et al., 2017). In contrast, inoculation and mulching reduced the root:shoot$_{\text{ratio}}$ (Study B), indicating that mulch was able to buffer drought conditions and improve plant growth. These buffering effects of mulch under drought may explain farmers' reports of early yield improvement after adapting to CA, despite a lack of measurable soil fertility improvements (Study A). As almost all soils in our studies (A, B, C) are below the defined critical limits of 2% SOC and 0.2% TN – or just slightly higher – required to enable crop growth (Landon, 2014; Parwada et al., 2022), this indicates that the low soil fertility constrains current agricultural management.

Microbial response to soil management practices

At different scales, microbial parameters responded more sensitively than soil chemical parameters to land use, providing early indications of improved soil function under CA. Microbial diversity (Shannon-Wiener and Simpson index) was higher in CA compared to W, while microbial biomass did not differ across land-use types (Figure 9). A decreasing trend for basal respiration and the metabolic quotient was observed from TA to CA to W, indicating increased microbial efficiency under CA (Study A). This increased efficiency is likely explained by reduced soil disturbance under CA, which promotes a more stable microbial habitat and higher residue inputs compared to TA (ZUBER & VILLAMIL, 2016; FENG et al., 2021; SINGH et al., 2021). Consequently, intensive tillage, reduced residue inputs, and monocropping under TA limit substrate diversity, such as root exudates and nutrient supply for microorganisms, thereby decreasing microbial diversity (SINGH et al., 2021).

The results of our laboratory experiments supported these observations, as microbial biomass did not differ between unmulched and mulched soils (Study B), indicating that CA primarily alters microbial community composition rather than increasing microbial biomass, but these responses depend on water and substrate availability (MBUTHIA et al., 2015; SINGH et al., 2021).

Enzyme activity responses to mulching depended on the enzyme type and on water availability. Under water optimum, the combination of inoculation and mulch increased potential BG activity, whereas mulch alone did not increase potential BG activity. This increase in enzyme activity reflects microbial responses to the combined availability of labile C and N derived from cowpea (ALLISON & VITOUSEK, 2005; STOCK et al., 2019; UWITUZE et al., 2023; JIANG et al., 2025). Although mulch alone did not increase potential BG activity, it likely supplied labile substrates supporting microbial growth and nutrient turnover (DU et al., 2022; NOOR UL et al., 2022; JIANG et al., 2025; WANG et al., 2025b). In contrast, increasing substrate affinity (K_m) and potential enzyme activity (V_{max}) of LAP in unmulched compared to mulched soils under both water conditions indicate a changing microclimate in the soil and resulting microbial adaptation to higher soil temperatures or N scarcity (STONE et al., 2012; RAZAVI et al., 2016a; STOCK et al., 2019; UWITUZE et al., 2023; LI et al., 2024b). This indicates an enhanced microbial N demand in unmulched soils with low OM content or nutrient availability (ALLISON & VITOUSEK, 2005; STOCK et al., 2019; UWITUZE et al., 2023; LI et al., 2024b).

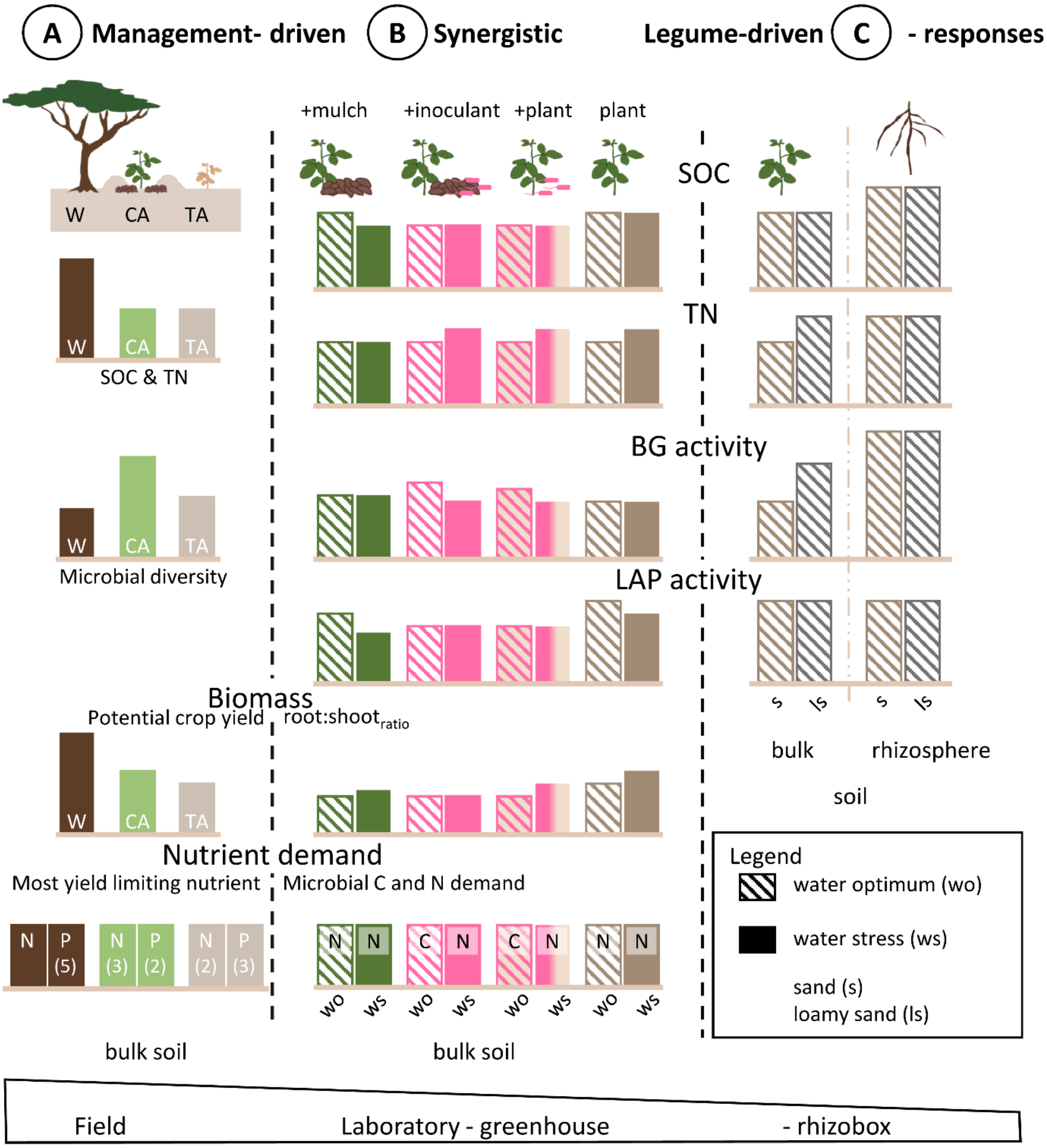

Figure 9: Management-driven (Study A and B), legume-driven (Study B and C) and synergistic responses (Study B) of soil properties across different spatial scales (bulk and rhizosphere soil) under field and laboratory conditions. Field study A includes three land-use types: woodland (W), conservation agriculture (CA) and traditional agriculture (TA). Different colours indicate different management practices across studies. Potential crop yield and most limiting nutrient refer to results of QUEFTS (Quantitative Evaluation of the Fertility of Tropical Soils), and the number below each nutrient represents the number of sites where that nutrient was limiting (Study A). Microbial C and N demand refers to enzymatic C:N ratios, where microbial demand is indicated by respective letter (Study B). SOC= soil organic carbon, TN= total nitrogen, BG= β-glucosidase, LAP= leucine-aminopeptidase.

3.2 Legume cultivation to enhance carbon and nitrogen in the soil

Legume N dynamics and inoculation effects

Inoculation of cowpea with a locally adapted *Bradyrhizobium*, from a neighbouring site in Namibia, altered soil N dynamics, microbial enzyme activities and enzymatic C:N ratios, demonstrating the inoculant's potential to improve N availability in low-fertility sandy soils (Study B; Figure 9). Inoculation increased soil TN and lowered C:N ratios, indicating improved N availability via N fixation and rhizodeposition (Study B). This observation is commonly reported in the literature (FUSTEC et al., 2010; PAUSCH & KUZYAKOV, 2018; SRIVASTAVA et al., 2025), suggesting that improved N fixation through inoculation of legumes can reduce N limitations under experimental conditions. This highlights the importance of N-fixing legumes in this region to improve N content in these sandy soils. Nitrogen was previously reported as the most yield-limiting nutrient in Namibian agricultural sites (DE BLÉCOURT et al., 2019), and was likewise identified as the most limiting nutrient under CA in our study (Figure 9). This was further supported by higher LAP enzyme activity in uninoculated bulk soil, suggesting high microbial N demand, likely due to limited N availability in the studied low-nutrient soils (Studies B and C). Inoculation, in contrast, reduced enzyme activity of LAP, suggesting reduced microbial N demand (Study B). This was further supported by different enzymatic C:N ratios between inoculated and uninoculated soils (Study B). Under optimum water conditions, uninoculated soils remained N-limited, whereas inoculated soils had improved N availability and thus shifted toward C-limitation (Study B; Figure 9). Lower LAP enzyme activity in rhizospheres than in bulk soil further reflected reduced microbial N demand near the N-supplying legume (Study C).

Legume-mediated effects in bulk soil with plant growth

Bulk soil BG and NAG enzyme activities increased with plant growth and were rather driven by substrate limitation and initial soil properties than direct plant influence (Study C; Figure 10). In contrast, bulk SOC and TN did not vary during plant growth in both soils (loamy sand and sandy soil). These constant bulk soil properties suggest that increased enzyme activities were driven by the microbial C demand due to variability of available C compounds (ALLISON et al., 2011; VERES et al., 2015; HOANG et al., 2016; GE et al., 2017; JAT et al., 2021) or indicate a diffusion of enzymes from the rhizosphere (VERES et al., 2015; WANG et al., 2023b). This increase in enzyme activity with ongoing plant growth might reflect higher microbial demand for easily available substrate during ongoing SOM turnover, leading to greater investments in enzyme production (BECKER &

HOLZ, 2021; JAT et al., 2021). Thus, bulk soil enzyme production is likely driven by nutrient limitation.

Legume-mediated rhizosphere processes

In the rhizosphere, cowpea cultivation sustained high activities of C- and N-acquiring enzymes (BG, NAG) during plant growth, resulting in spatial differentiation between rhizospheres, nodules and bulk soil (Figure 10). Rhizosphere BG and NAG enzyme activities were consistently higher than those in bulk soil. In both the loamy sand and sandy soil, enzyme activities were highest around roots and nodules, reflecting a resilient rhizosphere microenvironment (SICZEK et al., 2018; KAZMIERCZAK et al., 2020; WANKHADE et al., 2025) (Study C). As plants developed, root and nodule growth as well as root biomass increased, and enzyme activities became evenly distributed throughout the soil, reflecting root effects and rhizosphere inputs. Despite temporally stable enzyme activities in the rhizospheres, rhizospheric SOC increased, whereas TN remained stable over time. This indicates a constant supply of labile C via root exudation and rhizodeposition that maintained microbial activity and enzyme synthesis during plant growth (HOANG et al., 2016; GE et al., 2017; SUN et al., 2020; WANKHADE et al., 2025). Observed enzyme activities in our study were considerably higher than reported for other leguminous crops (LIU et al., 2017; MA et al., 2018a), highlighting cowpea's ability to mobilise nutrients and maintain microbial activity in low-nutrient soils.

At the surface of cowpea nodules, NAG activity slightly increased with plant growth, coinciding with constant TN content and increasing root biomass. This pattern reflects increased N availability from N fixation, which releases microbially available ammonium to the rhizosphere, stimulating microbial growth and enzyme production (SICZEK et al., 2018; LIU et al., 2025). Such increases in enzyme activities are relevant in N-limited soils, where microorganisms respond to small increases in available N (MA et al., 2018b; HUANG & HARTEMINK, 2020; LIU et al., 2022).

Nodule senescence at plant maturity stage only occurred in the sandy soil, where it represented a temporary labile OM input rich in C and N. This was reflected in higher BG activity around senescent nodules compared to root and nodule rhizospheres at cowpea maturity stage. Thus, nodule senescence temporarily increased available substrate, which was rapidly decomposed by microorganisms, as reflected by high enzyme activity (VERES et al., 2015; THILAKARATHNA et al., 2016; BLESH, 2019; KAZMIERCZAK et al., 2020; SUN et al., 2021; WANG et al., 2023b). This legume-driven input of C and N during nodule senescence represents an additional input beyond root exudation and N fixation, highlighting an important role of nutrient turnover in legume-soil interactions (Study C). These observed spatial and temporal patterns of enzyme activities in the rhizosphere show that cowpea creates rhizospheres characterised by continuous rhizodeposition and microbial decomposition (KUZYAKOV & BLAGODATSKAYA, 2015; GE et al., 2017), particularly in the loamy sand. Pronounced effects in the rhizosphere demonstrate that, despite low SOC stabilisation capacity of sandy soils, root-derived inputs can locally overcome substrate limitations.

Overall, cowpea cultivation increased SOC and TN contents in the rhizosphere and bulk soil under optimum water conditions (Studies B and C). These increases have been attributed to combined effects of root exudation, which stimulates microbial activity, and enhanced biological inputs from rhizobia, either native or inoculated (WICHERN et al., 2008; LIANG, 2020; LIU et al., 2022; KANTÉ et al., 2023; LIU et al., 2025).

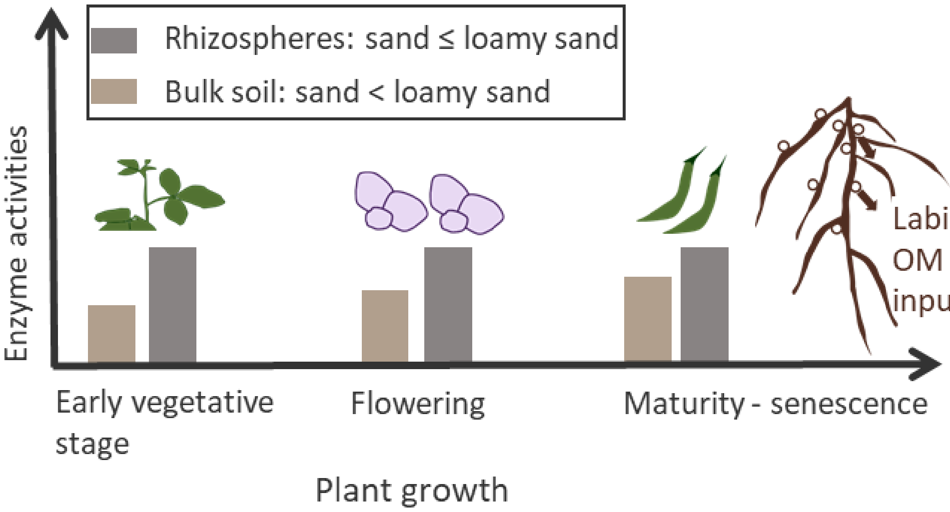

Figure 10: Overview of key results of the rhizobox experiment at different spatial (rhizosphere= grey color and bulk soil= brown) and temporal (plant growth) scales cultivated with cowpea in Mashare (loamy sand) and Ogongo (sand) soil related to Study C. OM= organic matter.

3.3 Site-specific conditions as predictor of management success

Combined findings from field and laboratory studies showed that the success of CA, including legume cultivation and mulching, in northern Namibia is strongly determined by soil texture and soil water availability coupled with temperature, which is also confirmed by other studies (ZUBER & VILLAMIL, 2016; HUANG & HARTEMINK, 2020; MALOBANE et al., 2020; GUJA & BEDEKE, 2024). These site-specific factors explain why CA resulted only in limited short-term improvements in soil fertility in the field, despite clear potential demonstrated under controlled conditions.

Influence of soil properties on enzyme activity and soil fertility

Across studies A - C, soil texture and initial nutrient status were key factors controlling microbial and enzymatic processes consistent with ABSHIBA et al. (2025). Given the underrepresentation of sandy soils in current research (COLUNGA et al., 2025), this dissertation contributes to a more comprehensive understanding of microbial processes in soils with high sand content (> 75%). As soil texture remains relatively unchanged over time, it influences other properties, such as nutrient-holding capacity, soil structure and pH (HUANG & HARTEMINK, 2020; ABSHIBA et al., 2025).

The loamy sand consistently had higher BG and NAG enzyme activities compared to the sandy soil, despite similar root biomass and root C and N content (Study C). This indicates that enzyme activities are influenced not only by the plant but also by soil properties. Soil properties, like texture or initial SOC and TN content, also shaped enzyme dynamics (Study C). Therefore, differences in enzyme activities reflect the capacity of less coarse-textured soils to stabilise SOM and support microbial activity. This linkage between texture and microbial activity is well documented and explained by SOM protection and enzyme adsorption on clay surfaces (ZIMMERMAN & AHN, 2010; VINHAL-FREITAS et al., 2017; ZHU et al., 2021; SHENG et al., 2022; ISLAM et al., 2025). Although both soils used in the study (loamy sand and sandy soil) belong to the sand texture class, small differences in the content of finer-textured particles already affected enzyme activities. This soil texture-induced enzyme stabilisation enhances enzyme persistence and promotes nutrient cycling. SHENG et al. (2022) demonstrated that BG activity persisted longer when adsorbed onto clay minerals, whereas it decreased faster when associated with primary silicate minerals typical for sandy soils, reflecting differences in adsorption capacity depending on soil texture (Study C). Additionally, coarse-textured soils have a limited capacity to form aggregates and stabilise C in the long term (ZUBER & VILLAMIL, 2016; HUANG & HARTEMINK, 2020; TAYLOR et al., 2021; COLUNGA et al., 2025). Thus, continuous and high organic inputs are required to at least maintain SOC and TN under semi-arid conditions in sandy soils (Study A). This was reflected in the highest SOC stocks and TN content at W, despite this land-use type having the highest sand content. This suggests that continuous organic inputs can increase or maintain SOC stocks and TN content, even in sandy soils under semi-arid conditions (Studies A and B).

Higher SOC and TN contents usually stimulate microbial activity and growth (SINSABAUGH et al., 2014; VINHAL-FREITAS et al., 2017), which consequently enhances enzyme synthesis and activity (ALLISON & VITOUSEK, 2005; WEI et al., 2019) and microbial functioning (Study C). While soil texture and initial soil properties strongly influenced enzyme and microbial activity at the bulk soil scale, these differences disappeared in the rhizosphere, where root-derived inputs temporarily balanced initial soil differences. With ongoing plant growth, cowpea was able to outbalance initial differences in SOC and TN content also in the bulk soil (Study C).

Effect of CA duration, temperature and water availability on soil fertility

Long-term studies on real agricultural sites from smallholder farmers are currently lacking. Consequently, reported effects of land management on soil fertility are inconsistent, especially in sandy soils. Although smallholder farmers implemented CA for more than six years within this study, SOC stocks and TN contents on agricultural sites (CA and TA) remained comparable (Study A). Long-term studies suggest that detectable increases in soil chemical properties and soil fertility require more than 10 years and continuous implementation of all CA principles (MBUTHIA et al., 2015; CORBEELS et al., 2020; THIERFELDER & MHLANGA, 2022; COLUNGA et al., 2025). This suggests that time since management adaptation and initial soil properties limit the benefits of CA for soil fertility (mulch and crop diversification) and that OM inputs were potentially insufficient to balance decomposition losses (DE BLÉCOURT et al., 2019; TAYLOR et al., 2021).

As another site-specific factor, temperature can limit SOC accumulation. A meta study by COLUNGA et al. (2025) observed that SOC accumulation under CA practices was mainly observed at sites with a mean annual temperature between 16-25 °C. Climate data for Study A were obtained from the nearest SASSCAL weather station (Alex Muranda Livestock Development Centre, ID 31203), providing online-accessible daily to annual weather records (SASSCAL WEATHERNET, 2025). Mean annual temperatures for 2023 and 2024 were within this reported range. However, measured maximum temperatures above 30 °C indicate that optimum temperature is exceeded during the cropping season in our study area (Study A). Here, it should be noted that due to technical failures of the weather stations, data are missing or incomplete. Observations of incomplete or missing data were also reported by TRISOS et al. (2023) and limit climate projections for Africa, especially for countries apart from South Africa and Kenya.

Apart from temperature, water availability emerged as a driver of management success. Water stress conditions restricted beneficial effects of mulch and inoculant under laboratory conditions (Study B). Whereas under water optimum conditions, legume cultivation resulted in more pronounced effects like SOC and TN increase across studies (Studies B and C), highlighting the importance of soil water availability. Thus, the lack of differences between TA and CA in field Study A indicate that soil water availability and potential microclimate alteration through mulching limit microbial activity and nutrient turnover in sandy soils (DENG et al., 2021; BOGATI & WALCZAK, 2022; QU et al., 2023). Further, both field and laboratory experiments showed that benefits of mulch on microbial parameters do not occur under water-limited or drought conditions. This was demonstrated by a decreased enzymatic C:N ratio under low soil water availability, indicating N limitation (Study B), which might explain similar TN content under field conditions between TA and CA (Study A). This suggested that drought reduced microbial activity, rhizobial effectiveness and N fixation, thereby altering microbial nutrient dynamics (QU et al., 2023; WANG et al., 2025b). This was demonstrated in Study B, where soil TN increased under water stress compared to water optimum conditions. This TN increase under water stress conditions likely reflected reduced microbial activity and plant N uptake, resulting in a drought-driven accumulation of N rather than enhanced N availability in the soil (THILAKARATHNA et al., 2016; LEITNER et al., 2017; ZHU et al., 2018; DENG et al., 2021). Inoculation may mitigate these negative drought effects, as indicated by higher soil TN under water-stress conditions in inoculated compared to uninoculated soils. Higher soil TN under water stress in inoculated soils indicates a potential N supply via symbiotic N fixation. This may reduce the dependence on water-driven mass flow of N to the root, which is limited under low soil water availability. This pattern aligns with other studies demonstrating the potential of legume inoculation to enhance soil N under drought and to improve plant drought tolerance through maintained photosynthetic activity (JABBOROVA et al., 2021; ÁLVAREZ-ARAGÓN et al., 2023; WANG et al., 2025a).

The results highlight the potential of management practices, such as legume cultivation and inoculation, for sustaining N availability in low-fertility soils under controlled conditions (pot experiment in the greenhouse and rhizobox), whereas microbial and enzymatic parameters show that water availability and soil microclimate regulate nutrient cycling (Study B), thereby limiting the benefits of CA under field-scale drought conditions (Study A and B). All studies synthesised indicate that water availability and initial soil properties were key drivers of management-driven responses (Studies A, B and C).

4 Conclusion and outlook

4.1 Conclusion

This dissertation enhances the understanding of nutrient dynamics in sandy soils under semi-arid conditions in northern Namibia. Nutrient dynamics were assessed at field and laboratory (pot and rhizobox) scales by investigating soil chemical and biological properties under different land-use types (conservation agriculture, traditional agriculture, woodland).

At field scale across land-use types, nutrient dynamics were shaped by reduced organic inputs and increased soil disturbance on agricultural sites compared to woodland, which exemplified near-natural conditions. Thus, agricultural sites experienced soil organic matter losses, as reflected by differences in soil fertility, resulting in higher predicted crop yields in woodland compared to agricultural sites. Although conservation agriculture has been practised for six years, neither soil organic carbon nor total nitrogen contents showed detectable increases compared to sites under traditional agriculture. This demonstrates that long-term implementation of conservation agriculture is necessary to observe measurable improvements in soil chemical properties, particularly in low-fertility soils with high sand content that limits nutrient retention capacity. Since soil organic carbon and total nitrogen losses were attributed to time since land-use conversion in other studies, estimating the age of agricultural fields could contextualise nutrient losses between traditional and conservation agriculture. Since traditional agricultural fields may be younger than conservation agricultural fields, this may partly explain the similar soil fertility observed under both agricultural management practices at our sites.

Our laboratory studies showed similar trends, where short-term mulching, as a principle of conservation agriculture, did not alter soil organic carbon or nitrogen dynamics. *Acacia* sp. wood chips, generated from bush encroachment, performed similarly to wheat straw mulch over the short term. Hence, *Acacia* sp. mulch represents an effective alternative to common mulch materials, especially in regions with biomass-limited agriculture and high risk of soil erosion, like in Namibia.

Microbial parameters, however, responded more sensitively to agricultural management. Higher microbial diversity and increased efficiency under conservation agriculture under field conditions suggest that biological properties are early indicators of management-induced changes. Those may lead to detectable shifts in chemical soil properties over time.

In the bulk soil, enzyme activities reflected the combined effects of initial soil properties, such as soil texture, and plant-driven inputs from the rhizosphere, influencing nutrient mobilisation. Despite low-nutrient status, cowpea cultivation induced high enzyme activities during plant growth, stimulating microbial activity. This demonstrates the potential of cowpea to mobilise and acquire nutrients, which even balanced initial differences in soil properties. To boost N dynamics, legume inoculation with locally adopted rhizobia increased and sustained soil nitrogen inputs and lowered soil C:N ratios, even under low water availability. This demonstrates the potential of inoculation to reduce nitrogen limitation for semi-arid agricultural systems. Soil enzymatic C:N ratios further demonstrated that inoculation reduced microbial nitrogen demand under water optimum conditions, shifting microbial limitations towards carbon. Under drought, these beneficial effects of inoculation were reduced, but soil microorganisms partially adapted by increasing substrate affinity. Low soil water availability reduced enzyme activities and limited nutrient turnover. However, this adaptive microbial response may still contribute to gradual improvements in soil fertility under environmentally challenging conditions in low-nutrient soils. Compared to mulching, water availability and legume inoculation were strong short-term drivers of nutrient dynamics, demonstrating the importance of regulating microbial activity and SOM turnover in semi-arid agricultural systems. Legume inoculation combined with mulching may induce synergistic effects, further enhancing soil fertility, as indicated under laboratory conditions.

In the rhizosphere, microbial processes were mainly legume-driven. Root exudation and biomass production sustained high carbon- and nitrogen-acquiring enzyme activities throughout cowpea plant growth. Particularly, nodule senescence at maturity stage emerged as a previously not investigated source of labile carbon- and nitrogen-rich OM input into the soil. These inputs induced short-term increases in enzyme activity, stimulating microbial decomposition pathways, highlighting the relevance of nodule senescence for fast nutrient mobilisation.

These findings indicate that continuous organic inputs and suitable mulch application rates are crucial for improving soil fertility in the long term. Within conservation agriculture, mulch remains a valuable and critical resource in semi-arid agricultural systems, whereas legume cultivation, with or without inoculation, provides a short-term source of microbially available nitrogen. While microbial responses indicate that conservation agriculture may gradually enhance soil fertility and crop yields over time, measurable changes in soil chemical properties require time and depend on continuous organic inputs and climatic conditions, like water availability.

Overall, the findings show that legume-driven processes and conservation agriculture interact with soil texture and water availability, shaping nutrient turnover and microbial processes across spatial scales in sandy soils in semi-arid regions like northern Namibia. While combining legume cultivation and conservation agriculture can intensify these effects, cowpea cultivation alone already substantially contributes to maintaining soil fertility under challenging conditions at the bulk soil scale. This was further demonstrated by plant-mediated inputs, with nodule senescence representing a relevant spatio-temporal OM input in the rhizosphere. By linking nutrient cycling from the rhizosphere to the field scale, these findings provide a basis for adapting soil management strategies under climate change and may help maintain soil fertility and food security in smallholder farming systems.

4.2 Implications for research perspectives

This dissertation contributed to field research in northern Namibia and to the understanding of potential effects of conservation agriculture on soil biogeochemical properties under field and controlled laboratory conditions. Although conservation agriculture did not significantly increase soil fertility, it indicates upward trends toward improved soil fertility over the long term compared with traditional agriculture.

In laboratory studies, legume cultivation and inoculation were strong short-term drivers of soil nitrogen availability. These findings indicate the need to test whether legume inoculation, combined with conservation agriculture, results in similar effects under field conditions. It would be interesting to investigate how inoculation might improve soil nitrogen status and enhance crop yields. Thus, future research should assess the applicability and effectiveness of these practices in smallholder farming systems, including participatory on-farm research, direct knowledge exchange, and identification of feasible, affordable and locally accepted management options. This is particularly relevant since smallholder farming systems represent a globally significant yet under-researched agricultural setting for food production.

Despite extensive global studies on conservation agriculture, there is limited synthesised research focusing on sandy soils, which dominate large parts of semi-arid regions. As land-use history appears to influence soil carbon and nitrogen dynamics, long-term monitoring sites should be established. Additionally, future study sites should be assessed for age, as age of agricultural sites was shown to be an indicator of carbon and nitrogen losses in other studies.

Since agricultural studies in Namibia are scarce, the established experimental field sites within the SUSTAIN project in northern Namibia (e.g., Mashare and Ogongo) should be retained to study the long-term effect of conservation agriculture and different management practices. Within this setup, crop rotations with and without legumes should be implemented, and mulch application rates, reflecting what smallholders can realistically supply, should be adapted. Such long-term studies could help identify alternative organic inputs and management strategies to improve soil fertility and support food security under semi-arid conditions.

Since soil texture and initial soil properties determined the success of soil management strategies, future research should investigate different textured soils and soil organic carbon fractions to understand better soil organic carbon stabilisation in soils of semi-arid regions. This would also help to guide decisions on whether other organic amendments are more suitable than mulch.

As responses of enzyme activities and soil chemical properties to legume cultivation and mulching differed across spatial scales, mechanistic studies remain essential. This dissertation demonstrated the pathway of organic matter input into the soil through legume nodule senescence for the first time. Future work should clarify how this process varies among legume species (different nodule types) and across soils with different textures and fertility, and how these variations influence soil carbon and nitrogen dynamics. Controlled laboratory experiments should further explore how legume-derived inputs, like nodule senescence and rhizodeposition, contribute to soil organic matter pools, stabilisation processes and potential hotspots of carbon and nitrogen retention. If laboratory studies indicate strong effects, field-scale studies should address how abiotic variability affects the agronomic relevance of this process.

Water availability was a driver of microbial responses in laboratory experiments. Therefore, future field and laboratory studies should implement climate-change scenarios to evaluate how drought and increasing climate variability affect microbial activity and nutrient-cycling responses under different amendments. This will help identify both limitations and potentials of those amendments to enhance soil fertility and agricultural resilience, and is especially important in the context of projected climate change impacts in Namibia, a sub-Saharan African country.

Finally, future work should aim to integrate these findings into a broader understanding of how legume-driven organic inputs, soil texture, and water availability interact to regulate carbon and nutrient dynamics in sandy soils in semi-arid regions to improve soil fertility. Addressing these research directions will improve soil fertility and food security under climate-change variability in semi-arid smallholder farmer agricultural systems.

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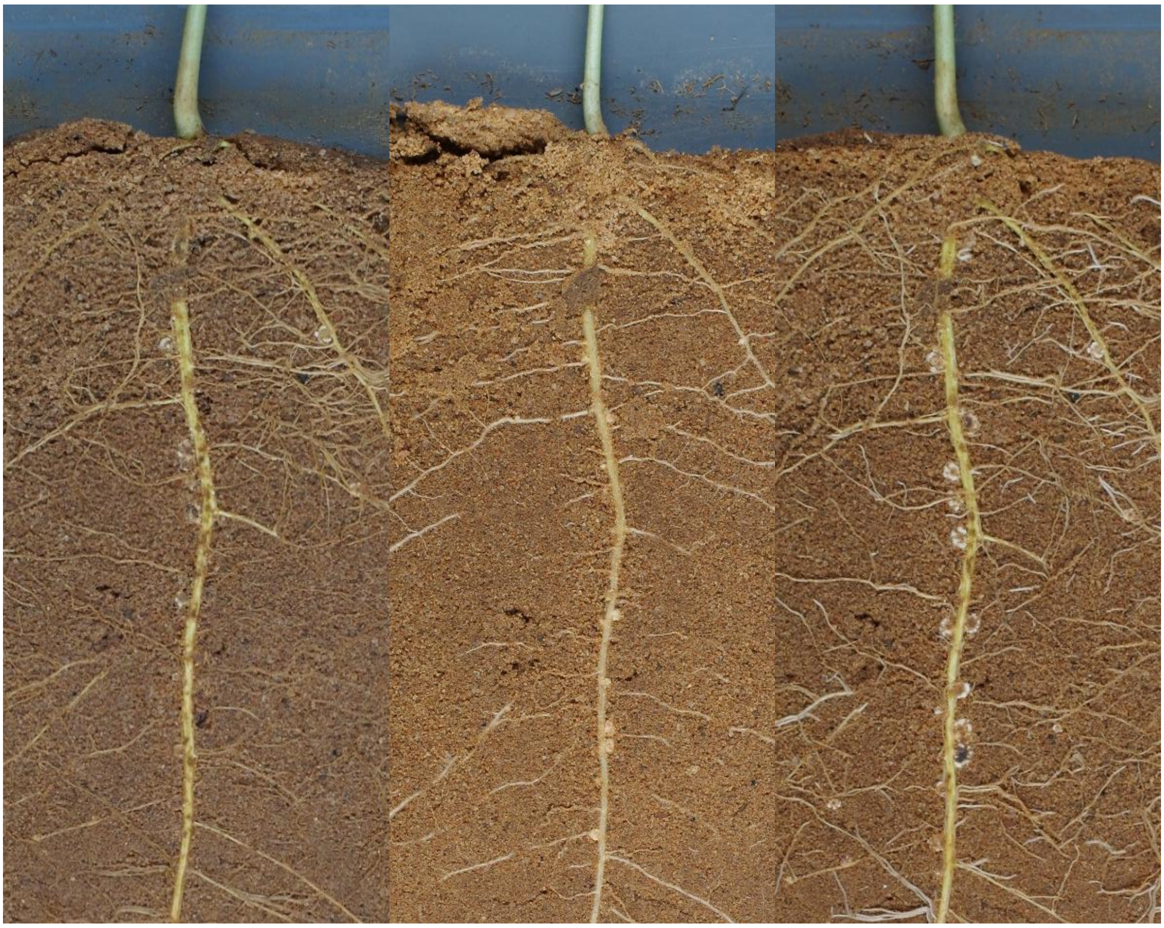

Study A:

Soil carbon, nitrogen and microbial responses under different land-use types in Northern Namibia

In preparation

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A Abstract

Conservation agriculture (CA) has been increasingly promoted as a climate-resilient management strategy, yet consistent evidence on its benefits for soil chemical and microbial properties, particularly in sandy soils, remain limited. We investigated soil chemical and microbial properties under three land-use types in the Kavango region of semi-arid Namibia. This included CA, traditional agriculture (TA), and woodland (W) sites in five replicates. Soils were sampled in two depths (0-10 cm and 10-20 cm) and analysed for soil organic carbon (SOC), total nitrogen (TN), pH, cation-exchange capacity, plant available phosphorus, microbial biomass and microbial community composition. Potential crop yields were calculated with the QUEFTS model. Our results showed no change in SOC stocks, TN or potential crop yields after six years of adaptation from TA to CA. In contrast, we observed an early trend toward a more diverse microbial community from W to TA to CA. These findings indicate that SOC accumulation and microbial community adaptation are time-intensive and site-specific. Particularly sandy soils in semi-arid regions are limited by the high sand content, but also by low nutrient availability. We conclude that continuous implementation of CA has the potential to enhance SOM and microbial efficiency, which may contribute to higher and more reliable crop yields and improved soil fertility in the long term.

Keywords

Semi-arid environment, microbial communities, conservation agriculture, QUEFTS

A1 Introduction

Globally, around 33% of the soils have been degraded and lost much of their soil organic carbon (SOC) and biological activity due to intensive agriculture, deforestation (AMELUNG et al., 2020; OKOLO et al., 2020; THIERFELDER & MHLANGA, 2022; SHIIMI & UCHEZUBA, 2025) and land-use conversion from native ecosystems (AMELUNG et al., 2020). In many regions, agricultural land is further expanded at the expense of woodlands (W) in order to cope with declining soil fertility (DE BLÉCOURT et al., 2019; AMELUNG et al., 2020). In sub-Saharan Africa (SSA), approximately 25% of soils are degraded, making the region particularly vulnerable to declining soil ecosystem functioning, reduced soil fertility, low crop productivity (CHIANU et al., 2011; AMELUNG et al., 2020; OKOLO et al., 2020), and increasing food insecurity under climate change (SIYAMBANGO et al., 2022; OSEWE et al., 2023).

Rainfed agricultural systems in SSA are especially vulnerable (AMELUNG et al., 2020; THIERFELDER & MHLANGA, 2022), as they are characterised by infertile sandy soils, low and erratic rainfall, and limited access to expensive fertilisers (BEKUNDA et al., 2010; CHIANU et al., 2011; PRÖPPER et al., 2013; DE BLÉCOURT et al., 2019; THIERFELDER & MHLANGA, 2022). Namibia is one of the most arid countries in SSA (SIYAMBANGO et al., 2022) but at the same time, about 98% of the population in northern Namibia depends on rainfed agriculture, with the Kavango region being one of the main agricultural areas (PRÖPPER et al., 2013; AWALA et al., 2019). Rainfed agriculture is characterised by intensive soil tillage, drought-resistant cereal crops such as pearl millet (*Pennisetum glaucum*), sorghum (*Sorghum bicolor*) and maize (*Zea mays*) with a few legume crops such as Bambara nut and cowpea, typically cultivated under low-input conditions (PRÖPPER et al., 2013; TAAPOPI et al., 2018; AWALA et al., 2019; SIYAMBANGO et al., 2022). Climate change is predicted to exacerbate these challenges with a projected temperature rise of 1.5 °C in northern Namibia expected to reduce rainfall and increase the frequency of extreme weather events (OSEWE et al., 2023; TRISOS et al., 2023).

To overcome these challenges and potentially restore soil fertility, conservation agriculture (CA) has been increasingly promoted as a climate-resilient and sustainable farming practice (KOPITTKE et al., 2019; THIERFELDER & MHLANGA, 2022; ARAYA et al., 2024; CHAPUNGU & ZHANDA, 2025). This practice is based on three principles: minimum soil disturbance, permanent soil cover and crop diversification (FAO, 2025). Therefore, improved water infiltration, higher soil organic matter (SOM) accumulation, higher crop yields, and enhanced soil biological activity are benefits of CA (SIYAMBANGO et al.,

2022; THIERFELDER & MHLANGA, 2022; ARAYA et al., 2024). In contrast, traditional agriculture (TA) often involves frequent tillage and limited crop diversification, which can reduce SOM and disturb soil microbial communities, potentially leading to declining soil fertility and crop productivity (SIYAMBANGO et al., 2022). Legume integration into CA further contributes to soil fertility through biological nitrogen (N) fixation (SIDDIQUE et al., 2012; HANSEN et al., 2017; THIERFELDER & MHLANGA, 2022; ARAYA et al., 2024). Despite these benefits, adaptation rates remain low, with less than 2% of cropland in SSA under CA and only 8% of smallholder farmers practicing all CA principles (ARAYA et al., 2024; SHIIMI & UCHEZUBA, 2025). By reducing soil disturbance and incorporating legumes, CA is expected to enhance soil C and N compared to TA, supporting soil biological activity and a more stable and efficient soil microbial community (THIERFELDER & MHLANGA, 2022). However, few studies have investigated how CA alters soil microbial communities and soil biochemical properties in dryland systems (MGANGA et al., 2024), and particularly in Namibia (TAAPOPI et al., 2018; THIERFELDER & MHLANGA, 2022). Moreover, a lack of long-term studies on real farmers sites limits the understanding of potential benefits of CA for soil fertility and crop productivity (SIYAMBANGO et al., 2022; COLUNGA et al., 2025) and sandy soils are underrepresented (DE BLÉCOURT et al., 2019; COLUNGA et al., 2025).

Microbial soil parameters respond faster to soil fertility changes than SOC or soil OM and are therefore sensitive indicators of land management effects (ZUBER & VILLAMIL, 2016; ADETUNJI et al., 2017; MALOBANE et al., 2020). Microbial parameters, like microbial biomass and community composition, provide insights into soil biological activity and efficiency (PABST et al., 2016). Microbial community structure is closely linked to SOM dynamics and long-term C storage (ZUBER & VILLAMIL, 2016; HALBRITTER et al., 2020). Consequently, microbial diversity is widely used as a key indicator for soil health and crop production (CHEN et al., 2024). A diverse and efficient microbial community enhances nutrient cycling, which can directly support crop productivity and enhances resilience against environmental stresses, like drought (CHEN et al., 2024). Despite these important functions, studies on microbial responses to CA under dryland conditions remain scarce (THIERFELDER & MHLANGA, 2022; MGANGA et al., 2024).

Here, we investigated the effect of long-term CA compared to TA and natural woodland (W) sites in the Kavango region in Namibia. Effects of land management on soil properties were assessed by comparing N pools, SOC content, metabolic quotient, and microbial community across land-use types. To link soil fertility and microbial activity to expected crop productivity, we applied the "Quantitative Evaluation of Fertility of Tropical Soils" (QUEFTS) model (JANSSEN et al., 1990; SATTARI et al., 2014). We hypothesised that 1) land management adaptation from CA to TA results in a C and N accumulation in soil and 2) CA would support a more diverse and efficient microbial community 3) thereby enhancing potential crop yields.

A2 Material and methods

A2.1 Site description and sampling

The study was conducted in the Kavango region of northern Namibia. Soil samples were collected from three different land use types, TA, CA and W (Appendix A 1). The area has a semi-arid climate (PRĂVĂLIE, 2016), with an annual temperature of 22.4 °C, and a mean annual rainfall of 450 - 550 mm occurring from November to March (AWALA et al., 2019). The soils have a sandy texture (at least 90% sand content). Sites under CA are managed by leader farmers trained by the GIZ (deutsche Gesellschaft für internationale Zusammenarbeit) according to the three CA-principles of the FAO. Minimum soil tillage was done with the ripper furrowing method, where furrows with a depth of approximately 30 cm are created that break through the soil. The fields have been under CA for at least 6 years with cowpea and pearl millet as rotation crops. For TA farmers do not irrigate, apply fertilizers nor herbicides as well as pesticides (GRÖNEMEYER et al., 2014), and prepare the soil with a plougher. Additionally, no soil cover is applied and monocropping is done (UCHEZUBA & MBAI, 2020; SIYAMBANGO et al., 2022), but we cannot exclude that occasional intercropping or crop rotations with legumes are implemented as adaptation strategies (SPEAR & CHAPPEL, 2018; TAAPOPI et al., 2018). Woodland area was sampled where the community forest was located, and some trees and no visible signs of cattle influence were considered as reference of near-natural conditions, dominated by shrubs, grasses and woody vegetation.

To cover a plot representative sampling, soil samples were taken with a shovel from 0-10 and 10-20 cm at 12 points at 5 m, 10 m, and 15 m from the plot centre in four cardinal directions. Each depth sample was mixed for one pooled sample per depth and plot. Sampling was conducted in April 2023 and March 2024. Samples were stored in plastic bags for shipping. When soil samples arrived, a part of the sample was immediately frozen until further analysis of microbial parameters.

A2.2 Soil chemical properties

The soil samples were sieved <2mm prior to laboratory analyses. The samples were analysed for pH (H_2O), SOC, total N (TN), exchangeable cations (Ca, K, Mg, Na), plant available phosphorous (P), and mineral N (N_{min}). For all analyses, we used the topsoil samples from both depths. Soil pH (H_2O) was measured in a 1:2.5 soil - to - water ratio. Soil C and N were analysed on milled and 105 °C oven-dried samples by dry combustion using an elemental analyser (varioMAX, Element Analyzer). Since pH was below 7, carbonates were not expected and we assumed that total C was equivalent to SOC. Exchangeable cations were extracted with 1M ammonium acetate and quantified by atomic absorption and atomic emission spectroscopy. We extracted plant-available P with a 0.5 M sodium bicarbonate solution at pH 8.5 (P - Olsen). P was determined photometrically. 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 (DR 5000 Lange Hach). To determine nitrate the same extract was used followed by analysis at high-performance liquid chromatography (Agilent Binary Technologies 1200 Series, Santa Clara, CA, USA).

A2.3 Soil physical properties

Soil bulk density was measured on samples taken in the plot centre with 100 cm^3 cylinders of the two depths. The soil core samples were oven-dried at 105 °C. The weight of the oven-dried samples was divided by the core volume to obtain the bulk density. Soil texture was determined using the sieving and sedimentation method based on the Köhn - pipette fractionation for mineral soils. A sieve tower (Retsch GmbH, Haan, Germany) was used to analyse the coarse fractions (2.0-0.063 mm), while a Sedimat 4-12 (Umwelt-Geräte-Technik GmbH, Muencheberg, Germany) was used to examine the fine fractions (<0.063 mm).

A2.4 Microbial biomass and basal respiration

Microbial biomass was determined using the chloroform fumigation method (VANCE et al., 1987). The fresh soil samples (25 g dry weight equivalent) set to a water content of 40% of the maximum water holding capacity were extracted in a 1:4 ratio of 0.05 M K_2SO_4. Another 25 g dry weight equivalent of fresh soil was fumigated with chloroform in a desiccator for 24 h and then extracted. The TOC/N analyser was used to analyse the extracts for dissolved organic C (DOC) and N (DON) content in a 1:2 dilution with K_2SO_4. Microbial biomass C (C_{mic}) and microbial biomass N (N_{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} and 0.54 for N_{mic}.

To estimate basal respiration (R_B) soil was pre-incubated at 40% of the maximum water holding capacity at 20 °C for one week. Basal respiration was analysed by measurement of CO_2 production using the flow-through method (Heinemeyer-device, MarCo Analytik, Hildesheim) over 24 h and detecting the CO_2 production with an infra-red gas analyser (CIRAS 3-DC). Airflow corrections were done to minimise variations and thus deviation. To evaluate raw data, we used the SIR-SBA 6.03 program.

A2.5 Microbial community analysis

For DNA extraction, 0.25 g of soil samples were suspended in high salt ammonium-buffer (3,5 M ammonium sulfate, 16.7 mM sodium citrate, 13 mM EDTA), and the Power Soil Pro Kit (Qiagen, Hilden, Germany) was applied including cell lysis by bead beating in a FastPrep bead beater at 4.0 ms^{-1} twice for 30 s with 30 s pausing between cycles. Extracted nucleic acids DNAs were further purified with High Prep PCR Magnetic Beads (Magbio Genomics, Galthersburg, MD, USA).

Amplification of variable region 4 of 16S rRNA genes was done with primers according to CAPOROSA et al. 2011 with one modification in the forward and two modifications in the reverse primer. Modifications given in bold letters: 16S new 515F (5'-GTG YCA GCM GCC GCG GTA A-3'), and 16S new 806R (5'-GGA CTA CNV GGG TWT CTA AT-3'). The first PCR was done on Biometra TProfessional Cycler (Analytik Jena GmbH+Co. KG, Jena, Germany) with 94 °C for 3 min, 25 cycles with 94° for 45 s, 50°C for 60s and 72° for 90 s, finished with 72°C for 5 min, using MolTaq DNA polymerase (Molzym GmbH & Co. KG, Bremen, Germany), 500 µM dNTPs (Life Technologies GmbH, Darmstadt, Germany), 50 µM of primers (Eurofins Genomics Europe, Ebersberg, Germany) and 20 ng of purified DNA templates. Barcoding of the products of first PCR was done with 20 different 10-mer barcodes attached to 5´-ends of primers individually per soil sample, with 10 cycles and conditions as above and 1 µl of template (PCR product odf 1st PCR). Barcode sequences are provided in Appendix A 2. Barcoded PCR-products were purified with High Prep PCR

Magnetic Beads before pooling into three pools with 20 samples each for library preparation, using NEBNext Ultra Express DNA Library Prep Kit (New England Biolabs GmbH, Frankfurt a.M., Germany).

After quality check using the High Sensitivity DNA Kit on Bioanalyzer 2100 (Agilent, Santa Clara CA, USA) and quantification with NEBNext Library Quant Kit for Illumina (New England Biolabs GmbH, Frankfurt a. M., Germany), these library pools were denatured, diluted to 10 pM and sequenced on MiSeq System (Illumina, Inc., San Diego, CA, USA) with MiSeq Reagent Kit v3 (600 Cycles), 250 bp paired-end sequencing. Sequencing resulted in 9.89 Mio reads identified passing filter reads with a quality score >=Q30 of 83.9%, that were further analysed bioinformatically.

A2.6 QUEFTS

We evaluated soil fertility of the soils using the QUEFTS model (JANSSEN et al., 1990; SATTARI et al., 2014). It estimates attainable crop yields under the assumption that N, P and K are the only nutrients limiting plant growth (DE BLÉCOURT et al., 2019). Among all cultivated crops, maize was the only crop covered by the model. The calculations included four steps: (1) Calculation of the potential soil supply of N, P and K using empirical relationships between soil chemical data and maximum nutrient uptake when no other nutrients or growth factors are limiting. Compliant with the information received from the farmers, we assumed no additional inputs of fertiliser. (2) Estimation of actual nutrient uptake from theoretical functions between the calculated potential supply of nutrients and actual plant uptake. (3) Estimation of yield ranges based on the estimated actual uptake of N, P and K, respectively, applying empirical equations for conditions, with and without limitations from other nutrients. (4) Estimation of the final predicted yield by combining the yield ranges calculated in step 3. Input data for the calculations included soil pH, as well as SOC and concentrations of exchangeable K, total N, and P-Olsen to 20 cm depth (average of the first two sampling depths.

A2.7 Calculations

To estimate SOC stock in soil, equation 1 was used as follows:

$$\text{SOC stock (t ha}^{-1}\text{)} = OC \times BD \times D \qquad (1)$$

Where OC is the SOC content (%) of a sampled depth, BD is the bulk density (g cm^{-3}) of soil at a sampled depth and D is the depth at which the soil sample was collected (cm).

A2.8 Statistical analyses

We used a linear mixed effect model with site as a random factor to test whether there are significant differences between land-use type and sampling depth. Sampling depth was defined as nesting factor followed by TukeyHSD post-hoc comparison if ANOVA showed significant differences. Assumptions of normality and homogeneity of variance were verified through visual inspection of the model residuals.

Statistical differences were accepted as significant at p-level <0.05. Statistical analyses and data visualisation were conducted in R (Version 4.5.0). Statistical differences between potential yield calculated with the QUEFTS model and SOC stocks were tested with ANOVA followed by TukeyHSD post-hoc comparison if ANOVA showed significant differences.

A3 Results

A3.1 Soil chemical properties and predicted crop yields

Soil OC stocks from 0-20 cm were higher in W compared to CA and TA ($p<0.05$), with a mean of 11.5 ±1.58 t ha^{-1} (Figure A 1a). SOC stocks in W were approximately double than in CA and TA, between which no difference was observed. The lowest (4.66 t ha^{-1}) and highest (9.19 t ha^{-1}) SOC stocks across all agricultural sites were both found in TA.

The highest predicted crop yields were found in W with a mean of 535 ±102 kg ha^{-1} decreasing to CA (331 ±119 kg ha^{-1}) and TA (262 ±102 kg ha^{-1}) (Figure A 1b). Predicted crop yield did not differ between CA and TA.

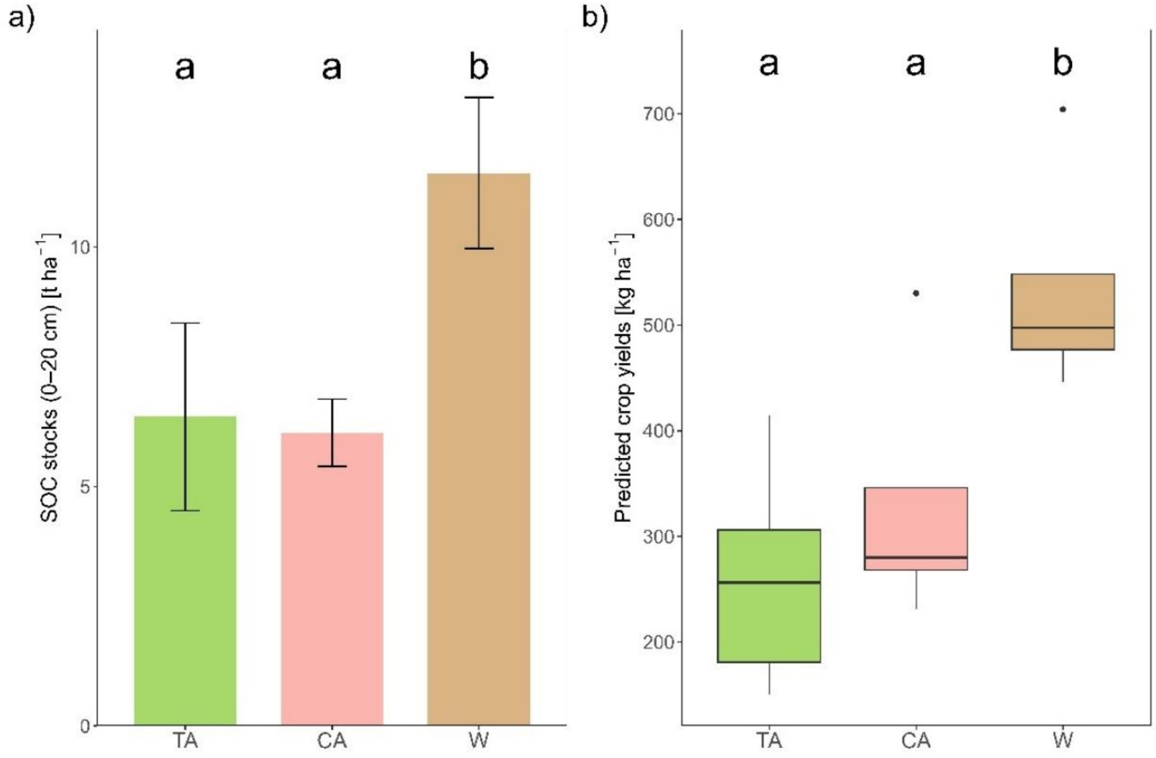

Figure A 1: a) Soil organic carbon (SOC) stock and b) predicted crop yield calculated with the QUEFTS model per land-use type. TA= traditional agriculture, CA= conservation agriculture W= woodland. Letters indicate significant differences between land-use types.

A similar pattern was observed for TN. No differences in TN were observed between depths in CA and TA. However, the upper depth of W had the highest TN content, approximately double than those of CA and TA ($p<0.05$; Figure A 2a). Under CA, N_{min} is slightly higher compared to TA, with mean values of 12.3 ± 6.11 mg kg^{-1} dw and 5.09 ±1.21 mg kg^{-1} dw, respectively (depth-averaged;

Figure A 2b). Across all land use types, N_{min} concentrations were within a similar range. Concentrations of P-Olsen were like N_{min} on a similar level among all land-uses (Figure A 2c). pH values differed only significantly between agricultural land use types (CA and TA) and W, with lowest pH in W (Figure A 2d).

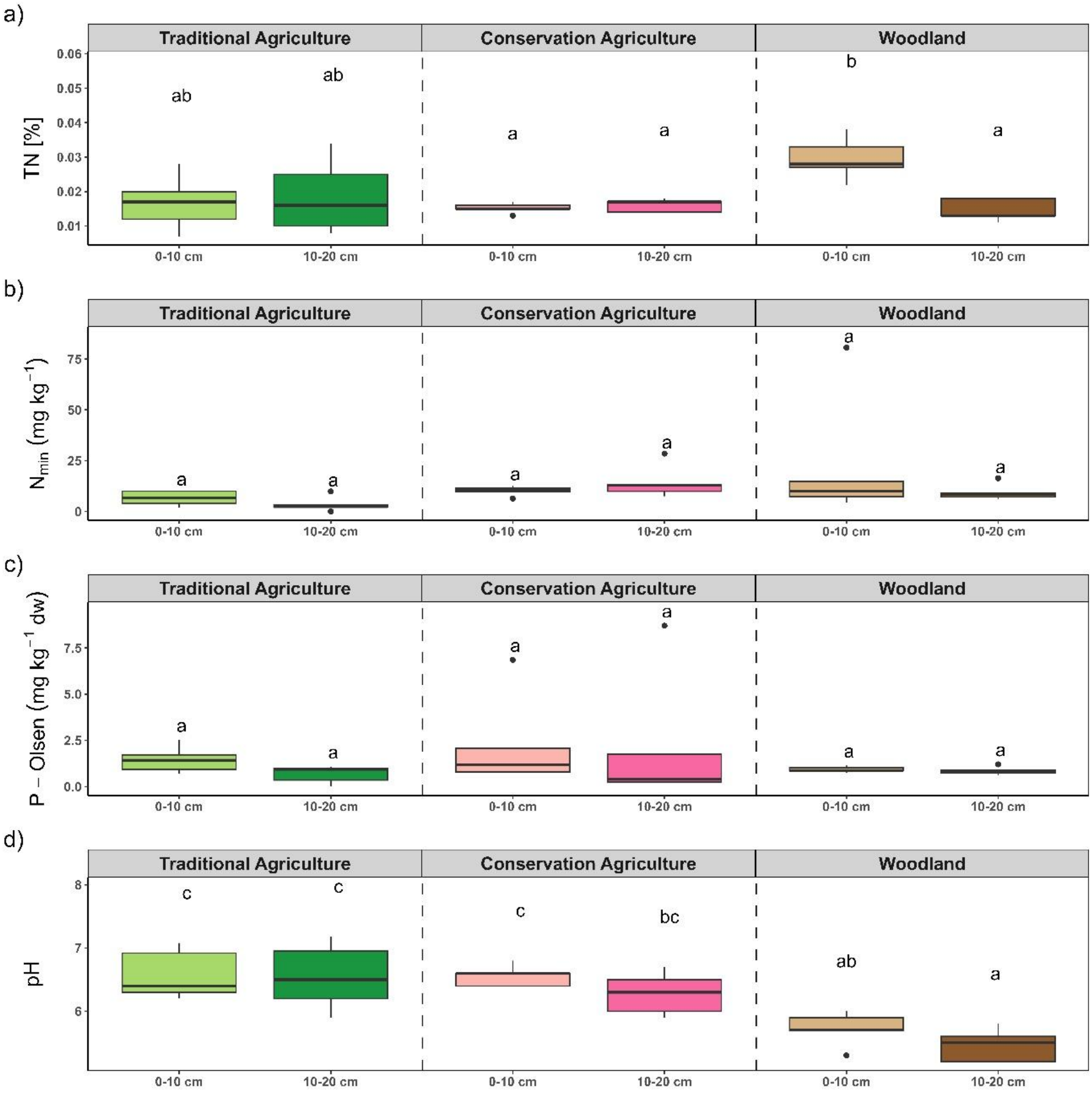

Figure A 2: a) Total nitrogen (TN), b) mineral nitrogen (N_{min}), c) plant available phosphorous (P-Olsen) and d) pH measured in water per land-use type and depth. Letters indicate significant differences between land-use types and soil depth.

A3.2 Soil microbial properties

Among all land-use types C_{mic}, did not differ ($p>0.05$; Figure A 3a). Generally, a trend of decreasing R_B was observed from W to TA to CA (Figure A 3b). Slightly higher R_B was found in TA. Under CA, the upper and lower depth differed, with higher RB in the first depth ($p>0.05$). Among land-use types and depths, qCO_2 showed no difference, but a slight decrease from TA to CA to W (Figure A 3c). For C_{mic} and R_B the highest variation was observed compared to CA and W.

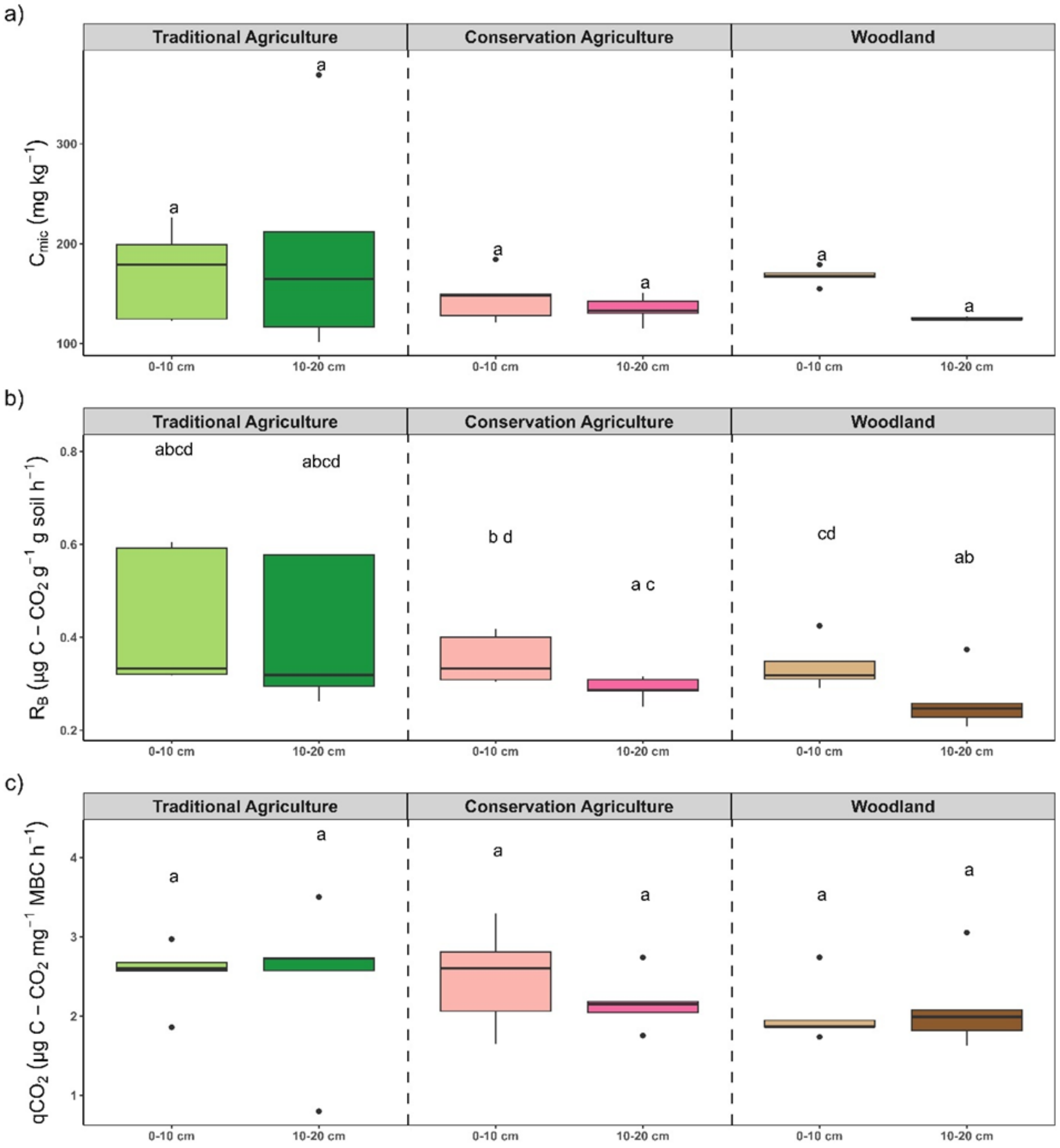

Figure A 3: a) Microbial biomass carbon (C_{mic}), b) basal respiration (R_B) and c) metabolic quotient (qCO_2) per land-use type and depth. Letters indicate significant differences between land-use types and soil depth.

A3.3 Microbial communities

Microbial diversity, expressed as the Shannon-Wiener index showed no statistical difference between land-use types and soil depths, though an indication of a trend was observed of higher microbial diversity in CA compared to W (Figure A 4a). The Simpson index, was higher in CA compared to W ($p < 0.05$; Figure A 4b), whereas no differences were observed between W and TA or TA and CA. A weak tendency towards greater diversity in CA compared to TA was observed. No depth effect was observed for both indexes.

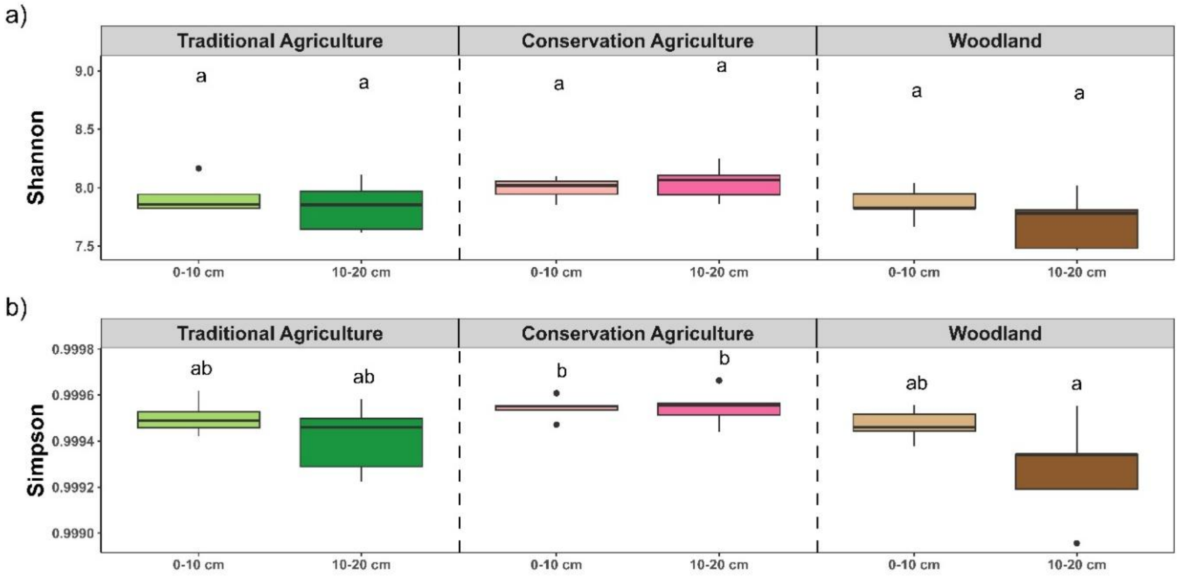

Figure A 4: Microbial diversity expressed as a) Shannon and b) Simpson index by land-use type and depth. Letters indicate significant differences between land-use types and soil depth.

Bray-Curtis community dissimilarity revealed differences between agricultural sites (CA and TA) and W ($p<0.05$; Figure A 5). Variation along non-metric multidimensional scale (NMDS) 2 was mainly associated with differences between soil depths of CA and TA. PCoA ordination resulted in a goodness of fit below 80% and is not shown.

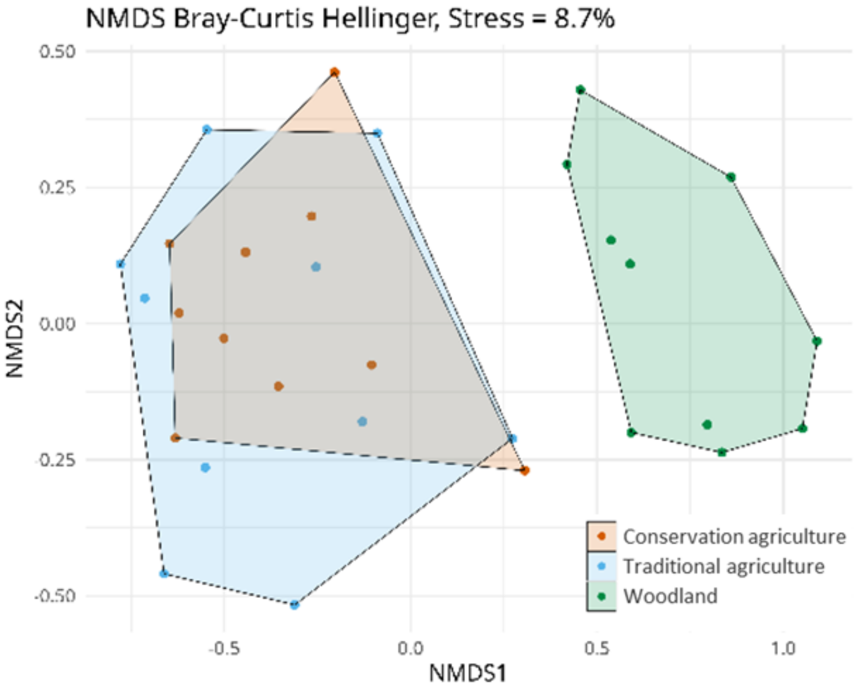

Figure A 5: Bray-Curtis community dissimilarity per land-use type. NMDS= non-metric multidimensional scale

A4 Discussion

A4.1 Impacts of site-specific conditions

Our results show differences between natural and agricultural land use types, with the highest SOC stocks and TN contents found in W, while CA and TA were comparable. This partially contradicts our initial hypothesis that CA would result in higher SOC and TN than TA, suggesting that measurable effects may require longer-term implementation under the given site-specific conditions (THIERFELDER & MHLANGA, 2022). Thus, duration of CA adoption appears to be a key factor and appeared as measurable in SOC and TN after more than 10 years and continuous implementation of all CA principles (MBUTHIA et al., 2015; CORBEELS et al., 2020; COLUNGA et al., 2025)

Apart from time, a study by MALOBANE et al. (2020) in south Africa indicates that the rate at which SOM increases is also influenced by site-specific soil conditions, climate, vegetation and the agricultural management practices. In our study, SOC stocks in W were approximately two times higher than in agricultural land-use, reflecting SOC loss through land use change. Similar patterns were reported by DE BLÉCOURT et al. (2019) in Namibian W and agricultural sites. They likewise found no clear differences among agricultural sites in SOC and TN stocks but increasing TN losses in 0-10cm and SOC losses in 10-20 cm related to age of agricultural sites. In addition, such losses can be attributed to reduced nutrient inputs, lower root turnover and greater soil disturbance (DE BLÉCOURT et al., 2019; TAYLOR et al., 2021; ARAYA et al., 2024). This confirms that land use is a major driver of SOC storage (SUH & TSHEKO, 2024). The lack of a clear difference between CA and TA suggests that potential benefits of CA are constrained by the sandy soil texture on our sites, which limits aggregate formation and long-term C stabilisation in general (GILLER et al., 2009; ZUBER & VILLAMIL, 2016; TAYLOR et al., 2021; COLUNGA et al., 2025). Limited SOC accumulation might also reflect microbial adaptation processes, where microbial communities become more efficient at decomposing OM, thereby reducing SOC accumulation (COLUNGA et al., 2025). Moreover, only few studies have examined soils with sand contents above 75% in semi-arid regions (COLUNGA et al., 2025), limiting the general understanding of CA effects under these conditions. In addition, the site-specific parameter temperature also drives SOC accumulation. A meta study by COLUNGA et al. (2025) showed that SOC increase under CA practices were mainly observed at sites with a mean annual temperature between 16 - 25 °C. Although mean annual temperatures for 2023 and 2024 were within the reported range for the selected weather station (SASSCAL WEATHERNET, 2025), measured maximum temperatures above 30°C indicate that optimum temperatures are exceeded during cropping season in our study area (Appendix A 3).

A4.2 Management and mulching effects

Continuous implementation of CA remains challenging, and type and amount of applied mulch likely influence nutrient dynamics and SOC accumulation (MASCIANDARO et al., 2004; EL-BELTAGI et al., 2022; MASETE et al., 2022; COLUNGA et al., 2025). Mulch amounts around 5 t ha^{-1} increased SOC (MASETE et al., 2022) compared to amounts of less than 2 t ha^{-1}, but these amounts are already considered critical for smallholder farmers (GILLER et al., 2009; MASETE et al., 2022). The availability of mulch materials (e.g. wheat straw., crop residues) remains a challenge. Due to low biomass production in semi-arid areas, smallholder farmers have to make a trade-off between mulching fields, feeding livestock or using it as construction material (GILLER et al., 2009; TAAPOPI et al., 2018; ARAYA et al., 2024). In addition, the risk of wind deportation of lightweight mulches (wheat straw) (APPIAH et al., 2023; AMIRI GHANAT SAMAN et al., 2025) or the loss of mulch due to soil fauna, e.g. termites, resulting in rather limited mulch effects (GILLER et al., 2009; ARAYA et al., 2024). Overall, these factors making it different for smallholder farmers to implement the CA principle mulching (GILLER et al., 2009; MASETE et al., 2022; PARWADA et al., 2022; ARAYA et al., 2024; CHAPUNGU & ZHANDA, 2025).

Moreover, residue quality - especially legume vs. grass residues - affects decomposition rates and nutrient release as well as SOC stocks (GILLER et al., 2009; TAYLOR et al., 2021; ARAYA et al., 2024). Since smallholder farmers implemented CA for more than six years within this study and SOC and TN on agricultural sites (CA and TA) were on the same level, we assume that mulch and crop diversification inputs from CA remain insufficient to balance the decomposition losses under semi-arid conditions (TAYLOR et al., 2021). However, the lack of detailed data on mulch application restricts further interpretation. Although we selected sites that fulfil criteria for TA, potential adaptations that include bushfires, intercropping with legumes, or legume inoculation cannot be excluded. Smallholder farms are heterogenous and adaptation strategies might be a reason for similar SOC and nutrient levels between CA and TA (VANLAUWE et al., 2019). Such adaptation strategies were documented for smallholder livestock farmers (SPEAR & CHAPPEL, 2018; SIRIRIKA et al., 2025).

Besides soil chemical properties, the potential yield calculated with the QUEFTS model was not higher in CA-managed fields compared to TA, contrary to our hypothesis. Nevertheless, a slight upward trend in potential yields under CA was observed. This is consistent with a meta-study by CORBEELS et al. (2020) who reported that yield improvements under CA in SSA are often small and mainly driven by mulching and crop rotations, but linked to enhanced soil fertility (DE BLÉCOURT et al., 2019). Overall, the calculated potential yield ranging from 151 t ha^{-1} to 535 t ha^{-1} remains below predicted crop yields from comparable land use types in Namibia (PRÖPPER et al., 2015; DE BLÉCOURT et al., 2019). We assume that this low range reflects the nutrient depletion caused by land-use conversion from W to agriculture. Additionally, low OM and nutrient inputs are insufficient to balance decomposition losses, resulting in a decline of soil fertility that cannot support higher crop yield potentials in smallholder farming systems under semi-arid conditions. This indicates that the current agricultural management is unsustainable und constrained by soil properties. In addition, all our sites are under the defined critical limits below 2% for SOC and below 0.2% for TN that constrain crop growth (LANDON, 2014; PARWADA et al., 2022). The uniformly low SOC and TN contents confirm the poor inherent fertility of these sandy soils.

A4.3 Microbial responses to land use type

Microbial responses to different land use partially confirm our second hypothesis that CA would support a more diverse and efficient microbial community, thereby enhancing potential crop yields. Microbial parameters, Cmic and RB did not differ between CA and TA in our study. This aligns with a study by MBUTHIA et al. (2015), who reported that CA does not necessarily increase microbial biomass but rather influences community composition and metabolic efficiency. Such functional shifts in microbial community composition can occur even without biomass changes, reflecting adaptation to reduced disturbance but also including crop rotations under CA (CHEN et al., 2024). These observations align with our data, which indicate an early trend towards increased microbial diversity from W to TA to CA. In addition, qCO_2 can be an indicator of differences in microbial metabolic efficiency. Tending to be higher qCO_2 values under TA indicate lower microbial efficiency, likely due to soil disturbance and reduced residue input. In contrast, CA systems enhance biological activity (THIERFELDER & MHLANGA, 2022; ARAYA et al., 2024) and thus promote microbial stability and efficiency, although this effect may be lowered by the relatively short duration of CA adoption and the sandy soils in our study. The decreasing trend of RB from TA to CA to W indicates gradual improvement of microbial activity and nutrient turnover under CA, consistent with findings from other Southern African soils (CORBEELS et al., 2020; THIERFELDER & MHLANGA, 2022). This is likely explained by higher soil disturbance, e.g. breaking up fungal hyphae, in TA systems (MANGALASSERY et al., 2015; OKOLO et al., 2020). In addition, higher microbial diversity in CA compared to W might also be caused by substrate quality or soil pH. Overall, our findings suggest that while CA has not yet increased SOC or TN, it shows early indications of enhanced microbial diversity and efficiency, indicating gradual development of improved soil functioning.

A5 Conclusion

We demonstrated that land-use adaptation from TA to CA on sandy soils under semi-arid conditions has not resulted in measurable increases in SOC stocks or TN content after six years. However, early indications of enhanced microbial diversity and efficiency suggest that CA may gradually improve soil fertility over time. These findings suggest that measurable improvements in soil biochemical properties under CA are highly site-specific and often require long-term implementation, particularly in sandy soils with limited C stabilisation potential. Although potential crop yields were not higher under CA, trends towards improved microbial functioning point to possible long-term benefits for soil fertility and productivity. Future studies should therefore focus on long-term field sites of smallholder farmers, particularly those with sandy soils, to better understand SOC dynamics, microbial processes, and their implications for sustainable agricultural systems in semi-arid environments in SSA.

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CRediT authorship contribution statement

Elisa Karina Albrecht: Writing – original draft preparation, Investigation, Conceptualisation, Formal analysis and investigation. Joscha N. Becker: Writing – review & editing, Conceptualisation, Methodology, Supervision. Judith Giel: Investigation, Writing – review & editing. Andrea Schaffrath: Investigation. Barbara Reinhold-Hurek: Writing – review & editing, Resources, Annette Eschenbach: Writing – Review & Editing, Conceptualisation, Supervision, Resources, Funding acquisition.

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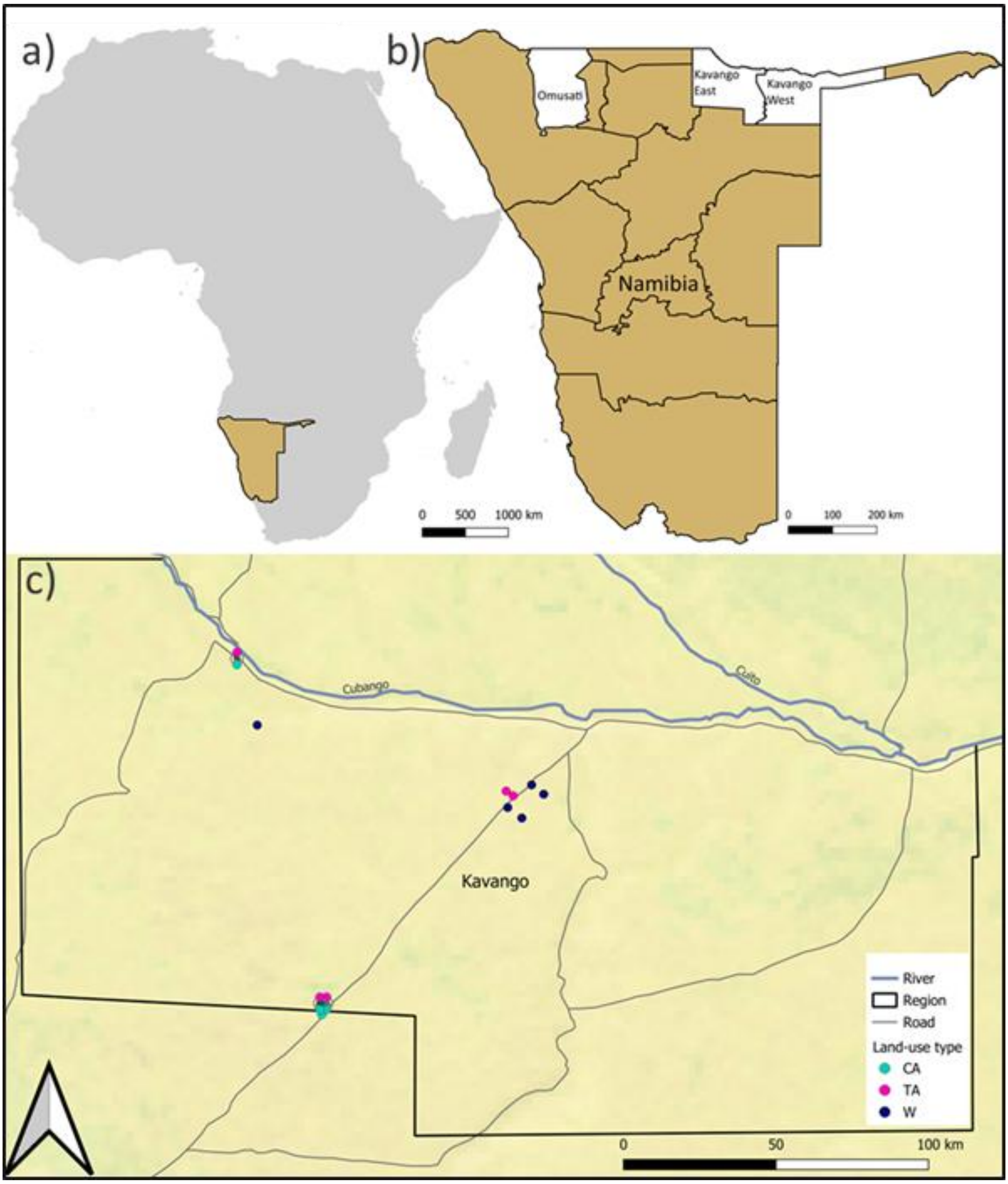
A7 Appendix



Appendix A 1: a) Location of Namibia in sub-Saharan Africa (brown filling) and b) location of the major agricultural regions Kavango and Omusati in northern Namibia, c) sites within the Kavango region under conservation agriculture (CA), traditional agriculture (TA) and woodland (W).

Appendix A 2: Barcodes for amplicon sequencing.

Name	Sequence
BC87-16SF	GCAGCGAGTAGTGYCAGCMGCCGCGGTAA
BC88-16SF	TACTCGCTATGTGYCAGCMGCCGCGGTAA
BC89-16SF	AGCATCGATGGTGYCAGCMGCCGCGGTAA
BC90-16SF	CATCGCTCGAGTGYCAGCMGCCGCGGTAA
BC91-16SF	GTCTCACTGTGTGYCAGCMGCCGCGGTAA
BC92-16SF	TGAGACGTATGTGYCAGCMGCCGCGGTAA
BC93-16SF	AGACTAGTCTGTGYCAGCMGCCGCGGTAA
BC94-16SF	CGAGATCTCTGTGYCAGCMGCCGCGGTAA
BC95-16SF	GCGAGTACGAGTGYCAGCMGCCGCGGTAA
BC96-16SF	TCACTGTGCGGTGYCAGCMGCCGCGGTAA
BC97-16SF	ATCTCGCAGTGTGYCAGCMGCCGCGGTAA
BC98-16SF	CACGTGTAGCGTGYCAGCMGCCGCGGTAA
BC99-16SF	GCTCAGTGAGGTGYCAGCMGCCGCGGTAA
BC100-16SF	TCATGTGTGTGTGYCAGCMGCCGCGGTAA
BC101-16SF	AGTATGTCGTGTGYCAGCMGCCGCGGTAA
BC102-16SF	CTACACAGAGGTGYCAGCMGCCGCGGTAA
BC103-16SF	GAGAGCACTAGTGYCAGCMGCCGCGGTAA
BC104-16SF	TAGCTATAGCGTGYCAGCMGCCGCGGTAA
BC105-16SF	ACTATGCGTAGTGYCAGCMGCCGCGGTAA
BC106-16SF	CTCGAGCATCGTGYCAGCMGCCGCGGTAA
BC107-16SF	GATCATCAGCGTGYCAGCMGCCGCGGTAA
BC87-16SR	GCAGCGAGTAGGACTACNVGGGTWTCTAAT
BC88-16SR	TACTCGCTATGGACTACNVGGGTWTCTAAT
BC89-16SR	AGCATCGATGGGACTACNVGGGTWTCTAAT
BC90-16SR	CATCGCTCGAGGACTACNVGGGTWTCTAAT
BC91-16SR	GTCTCACTGTGGACTACNVGGGTWTCTAAT
BC92-16SR	TGAGACGTATGGACTACNVGGGTWTCTAAT
BC93-16SR	AGACTAGTCTGGACTACNVGGGTWTCTAAT
BC94-16SR	CGAGATCTCTGGACTACNVGGGTWTCTAAT
BC95-16SR	GCGAGTACGAGGACTACNVGGGTWTCTAAT
BC96-16SR	TCACTGTGCGGGACTACNVGGGTWTCTAAT
BC97-16SR	ATCTCGCAGTGGACTACNVGGGTWTCTAAT
BC98-16SR	CACGTGTAGCGGACTACNVGGGTWTCTAAT
BC99-16SR	GCTCAGTGAGGGACTACNVGGGTWTCTAAT
BC100-16SR	TCATGTGTGTGGACTACNVGGGTWTCTAAT
BC101-16SR	AGTATGTCGTGGACTACNVGGGTWTCTAAT
BC102-16SR	CTACACAGAGGGACTACNVGGGTWTCTAAT
BC103-16SR	GAGAGCACTAGGACTACNVGGGTWTCTAAT
BC104-16SR	TAGCTATAGCGGACTACNVGGGTWTCTAAT
BC105-16SR	ACTATGCGTAGGACTACNVGGGTWTCTAAT
BC106-16SR	CTCGAGCATCGGACTACNVGGGTWTCTAAT
BC107-16SR	GATCATCAGCGGACTACNVGGGTWTCTAAT

Appendix A 3: Monthly weather data from the Alex Muranda Livestock Development Centre (Station ID: 31203) from Weathernet SASSCAL. Temperatures are monthly averages. (SASSCAL WEATHERNET, 2025)

Year	Month	Temperature [°C]	Minimum Temperature [°C]	Maximum Temperature [°C]	Precipitation (total) [mm]	Mean annual Temperature [°C]
2023	January	n.a.	n.a.	n.a.	n.a.	
2023	February	n.a.	n.a.	n.a.	n.a.	
2023	March	23.9	14.0	36.1	65.0	
2023	April	22.8	10.0	34.1	0.2	
2023	May	20.6	7.6	32.8	5.3	
2023	June	16.3	-0.2	32.0	0.0	
2023	July	15.5	-4.0	30.1	0.0	
2023	August	19.6	-5.6	36.2	0.2	
2023	September	24.0	7.6	37.9	7.4	
2023	October	28.4	11.5	40.4	5.2	
2023	November	27.8	16.8	39.5	63.6	
2023	December	25.8	13.1	36.9	76.2	
2023						22.5 ± 4.2
2024	January	24.4	15.7	37.4	87.4	
2024	February	n.a.	n.a.	n.a.	n.a.	
2024	March	n.a.	n.a.	n.a.	n.a.	
2024	April	20.9	8.4	34.6	0.0	
2024	May	18.9	3.5	33.8	0.0	
2024	June	15.4	-0.9	31.1	0.0	
2024	July	16.0	-1.5	32.9	0.0	
2024	August	19.8	4.1	34.4	8.9	
2024	September	25.4	5.9	38.8	0.0	
2024	October	26.1	10.3	40.0	0.0	
2024	November	26.3	13.6	38.6	51.2	
2024	December	25.8	13.6	39.0	39.3	
2024						22 ± 4

Study B:

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

Submitted

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B Abstract

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, yet their effects on soil biogeochemical properties in semi-arid regions remain poorly understood. 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 northern Namibia. Effects of different mulches (wheat straw, wood chips, 1:1 mixture of both), inoculation with a locally adapted Bradyrhizobium and soil water conditions on soil biogeochemical properties, including SOC, N, pH, microbial biomass and enzyme kinetics (β-glucosidase, chitinase, leucine-aminopeptidase) were investigated. 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 strongly decreased enzyme activities by up to 64%, indicating water-dependent benefits that 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. Our findings highlight cowpea inoculation as an effective strategy to improve soil N, while mulching provides additional benefits only under favourable water conditions. We conclude that combining inoculated 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

B1 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 (Soropa et al. 2019; Shiimi and Uchezuba 2025; 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; Thierfelder and Mhlanga 2022; Araya et al. 2024). 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 (Thierfelder and Mhlanga 2022; Chapungu and Zhanda 2025). 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 (Mupangwa et al. 2012; Masete et al. 2022), 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 (El-Beltagi et al. 2022; Thierfelder and Mhlanga 2022; Araya et al. 2024), 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 (Masete et al. 2022; Parwada et al. 2022; Araya et al. 2024; Chapungu and Zhanda 2025). 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; Gogoi et al. 2018; El Sabagh et al. 2020). Legumes also contribute high-quality organic matter (OM) and root exudates that improve nutrient availability for following crops (Siddique et al. 2012; Hansen et al. 2017; Becker et al. 2024). 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 (Deaker et al. 2004; Lupwayi et al. 2011; Rasche et al. 2023; Chen et al. 2024; Figiel et al. 2025). 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; Razavi et al. 2016b; Kuzyakov and Razavi 2019; Liu et al. 2024b; Jiang et al. 2025) 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 (Tian et al. 2020; Islam et al. 2025), 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. Here, we investigated the combined effects of water availability, mulch, and rhizobial inoculation 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.

B2 Material and Methods

B2.1 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). 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. 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 (CaCl2) of 5.38.

B2.2 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. 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 (WC), plus wheat straw (WS), and plus a 1:1 combination of both (WC+WS). Mulch types differed in substrate quality, whereas WC mulch has a lower C:N ratio compared to WS (B7 Supplementary Material 1). 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 of a locally adapted *Bradyrhizobium* sp. strain 26-nodO 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 UNAM campus in Ogongo at a neighbouring 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. 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).

B2.3 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 <2mm and frozen until further laboratory analyses.

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).

B2.4 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), 1h (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 (Equation 1; Michaelis and Menten (1913)), which describes nonlinear saturation curves and the catalytic enzyme activity was calculated after Moscatelli et al. (2012) Equation 2:

Equation 1 $$v = \frac{V_{max} \times [S]}{K_m + [S]}$$

Equation 2 $$K_a = \frac{Vmax}{K_m}$$

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 (Equation 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:

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

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.

B2.5 Statistical analyses

All calculations and statistical analyses were done using RStudio (Version 4.5.0) using 'lme4' package for statistical analyses, 'outliers' package to detect outliers based on the Dixon test ($p<0.05$), and 'ggplot2' 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 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. 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, t-test was done. To test for significant differences of SOC, TN, SOC:N ratio and

C_{mic}, respectively, we used the same statistical analyses and p-values ≤ 0.1 were declared as tendencies of significance.

B3 Results

B3.1 Soil chemical and microbiological properties

Under water-optimum conditions, SOC was affected by plant and inoculation, while TN showed a tendency to be influenced by inoculation. The C:N ratio was affected by inoculation and the interaction between plant, inoculation and mulch under both water conditions (B7 Supplementary Material 2). Under water stress conditions, SOC was influenced by mulch, whereas TN was affected by plant and inoculation (B7 Supplementary Material 2).

The SOC content was approximately 20% higher in planted treatments with WC and WS application under water optimum compared to water stress conditions (Figure B 1). These treatments also showed higher SOC contents than the unplanted unmulched treatment. Under water optimum conditions, TN was higher in inoculated (0.31 ±0.007 mg g^{-1}) compared to planted (0.279 ±0.009 mg g^{-1}) treatments (Figure B 1b). Especially inoculated with WC and WS treatment compared to planted treatment tended to be higher ($p \leq 0.1$; Figure B 1b). Under water stress, TN increased in the planted treatment with WS (+13.91%), in the unmulched inoculated (+19.89%) and in the inoculated WC+WS treatment (+16.67%) compared to water optimum conditions ($p<0.05$; Figure B 1b). The soil C:N ratio decreased under water stress in planted treatments with WC (-18%), WS (-36%) and WC+WS (-24%) compared to water optimum conditions ($p<0.05$; Figure B 1c). Similar decreases were observed in the inoculated unmulched (-27%), WS (-13%) and WC+WS (-20%) treatments ($p<0.05$). Inoculation tended to lower the C:N ratio under both water conditions ($p<0.05$; Figure B 1c).

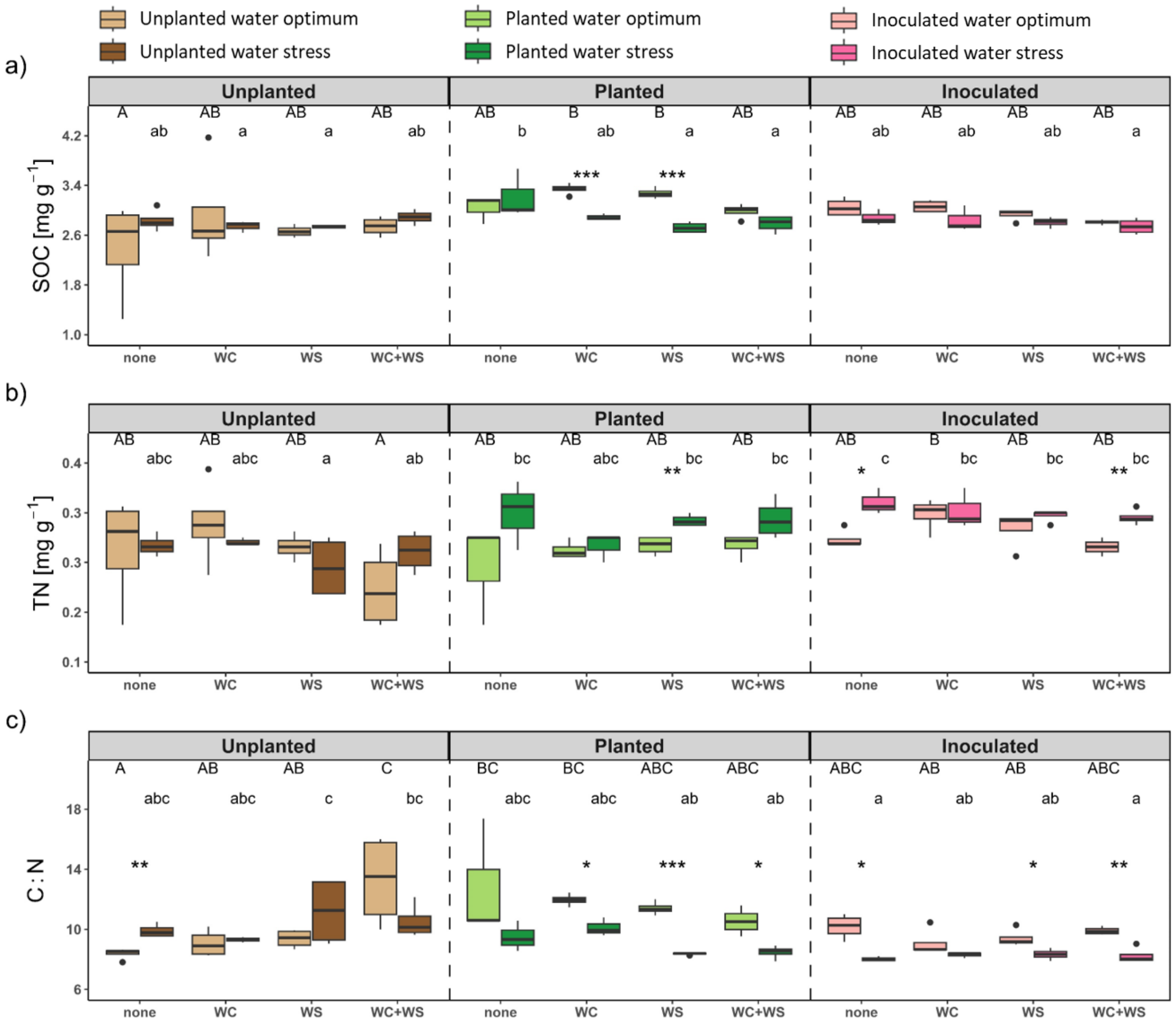

Figure B 1: a) Soil organic carbon (SOC) content (mg g^{-1}), b) total nitrogen (TN) content (mg g^{-1}), and c) soil C:N ratio in unplanted, planted and inoculated treatments (n = 4) with applications of WC = wood chips, WS = wheat straw, and WC+WS = wood chips and wheat straw mulch. Different letters indicate significant differences according to ANOVA, 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

The response of C_{mic} differed between water optimum and stress conditions. 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, affected C_{mic} (B7 Supplementary Material 2).

Particularly, with WS and WC+WS mulch in the planted treatment, C_{mic} decreased ($p<0.05$; Figure B 2). Under water stress conditions, inoculation tended to decrease C_{mic}, whereas WC mulch increased it ($p < 0.05$). The highest C_{mic} were observed in planted treatments without mulch (+50.41%) and with WC addition (77.25%) under water stress conditions.

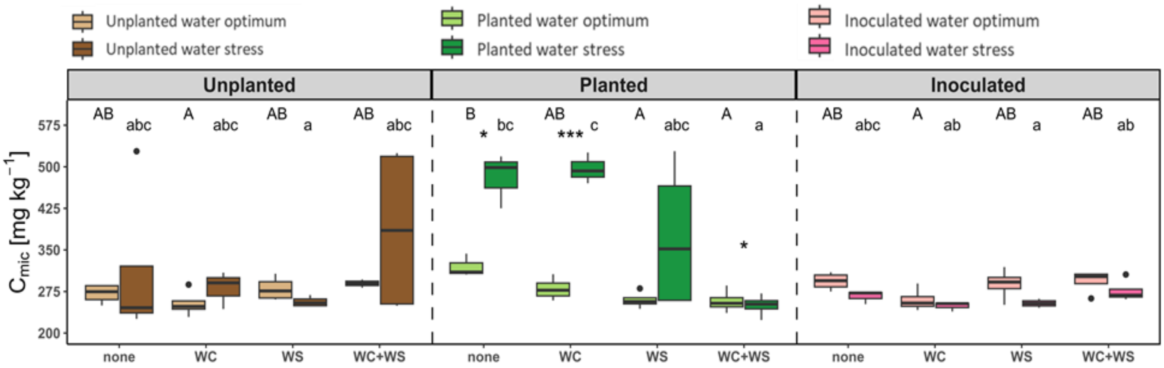

Figure B 2: Microbial biomass carbon (C_{mic}) in unplanted, planted and inoculated treatments (n = 4) with applications of WC = wood chips, WS = wheat straw, and WC+WS = wood chips and wheat straw mulch. Different letters indicate significant differences according to ANOVA, where capital letters show 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

B3.2 Soil enzyme kinetics

Across all enzymes, V_{max} of BG and NAG was approximately ten times higher than that of LAP (Figure B 3). Under water optimum conditions, plant, inoculation and their interaction with mulch affected V_{max} of BG, whereas LAP was affected by plant and inoculation as well as mulch. No treatment effects were observed for NAG (B7 Supplementary Material 3).

Under water optimum conditions, V_{max} of BG was higher in inoculated treatments (without mulch and with WC addition) compared to the other treatments ($p<0.05$; Figure B 3a). Under water optimum conditions V_{max} of BG was up to 143% higher than under water stress conditions for inoculated and WC mulched treatments, with the combination of WC and inoculation showing the highest activity (140 ± 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 (Figure B 3b). Under both water conditions, V_{max} of LAP decreased with mulch (Figure B 3c). Overall, V_{max} of LAP was highest in planted treatments and under water optimum conditions for some mulch treatments – no mulch, WC (+178%) and WS (+159%). For the inoculated treatment, only WC application differed significantly between water optimum and water stress conditions.

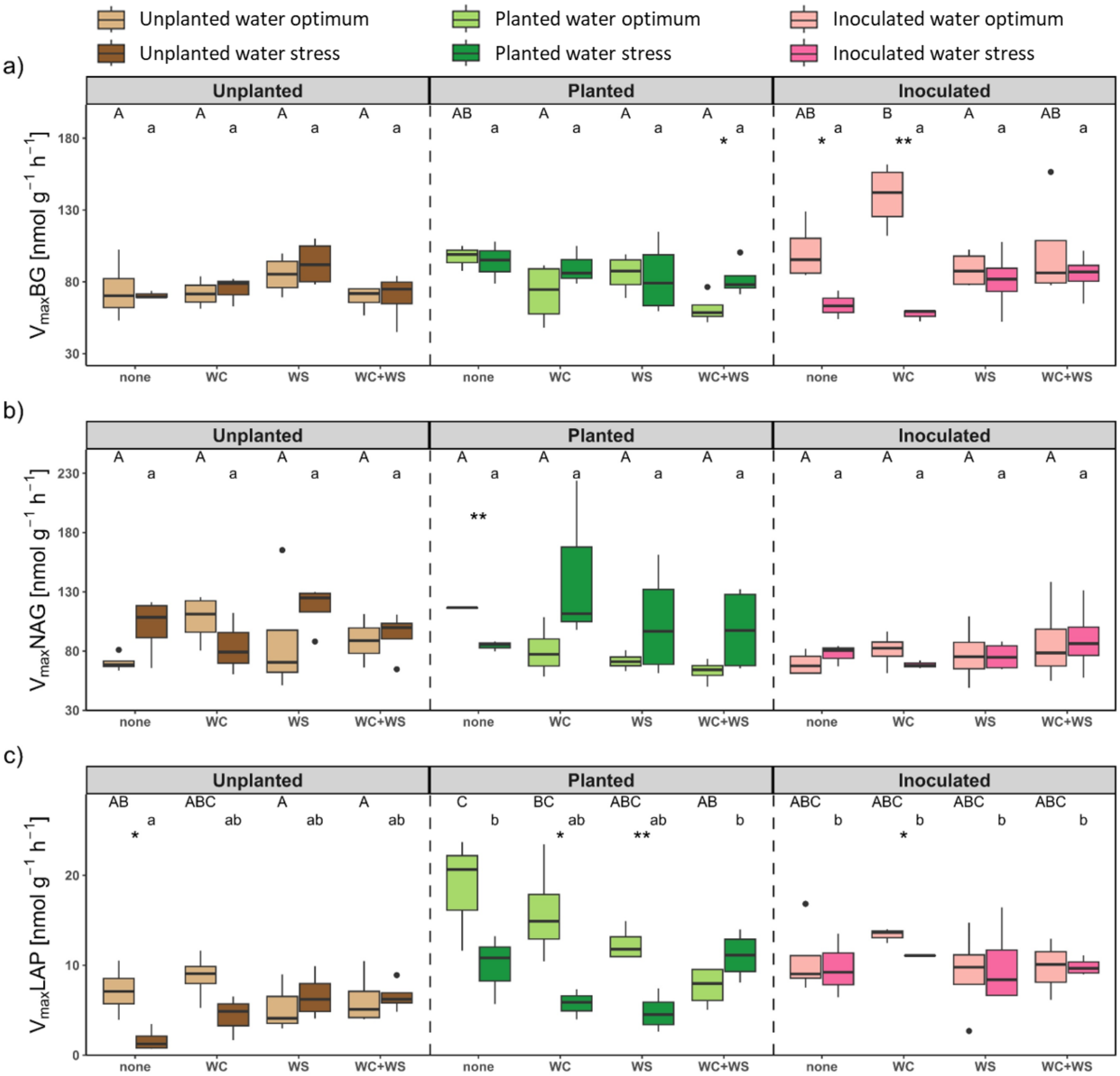

Figure B 3: a) Enzyme activity Vmax of ß-glucosidase (BG), b) chitinase (NAG), and c) leucine-aminopeptidase (LAP) in unplanted, planted and inoculated treatments (n = 4) with applications of WC = wood chips, WS = wheat straw, and WC+WS = wood chips and wheat straw mulch. Different letters indicate significant differences according to ANOVA, 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

Across all enzymes, K_m for NAG was approximately three times higher than that of BG and LAP (Figure B 4). Under water optimum conditions, plant, inoculation and their interaction with mulch affected K_m of BG and LAP, whereas also their interaction affected K_m of LAP (B7 Supplementary Material 4). No treatment effects were observed for NAG (B7 Supplementary Material 4). Treatment effects were less pronounced under water stress conditions, only K_m of LAP was affected by plant and inoculation, as well as the interaction with mulch.

Under water stress conditions, K_m of BG was lower in the inoculated WS (+181%) treatment compared to water optimum conditions, while mulch increased K_m under water optimum conditions ($p<0.05$; Figure B 4a). Under water stress conditions, K_m of BG was lower in inoculated treatments. For NAG, except the unplanted WS treatment, where K_m of NAG was up to 179 % higher under water stress conditions no effects were observed (Figure B 4b). Mulch decreased K_m of LAP under both water conditions, except the planted WC+WS and inoculated WC treatments under water stress conditions ($p<0.05$; Figure B 4c). 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.

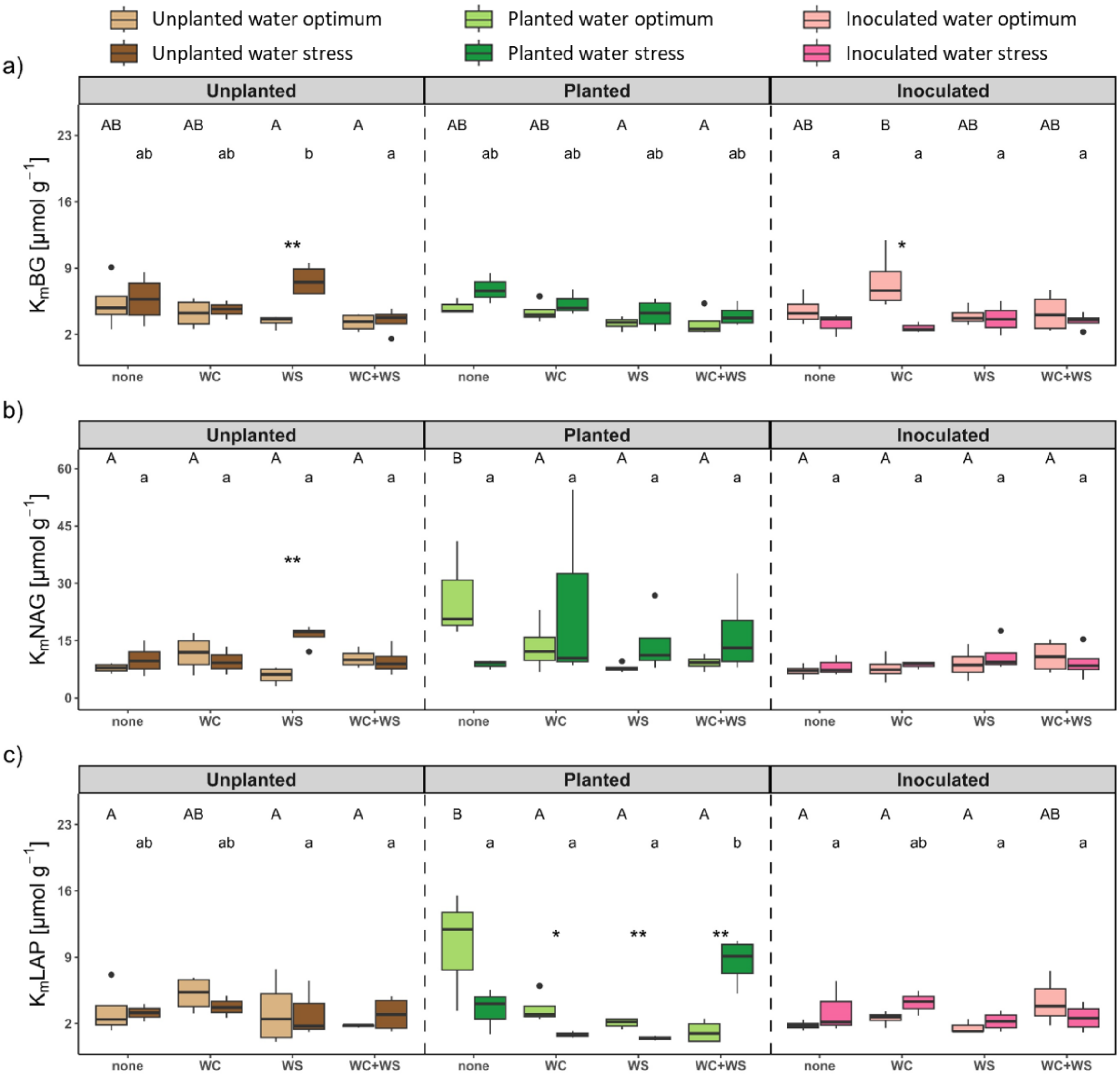

Figure B 4: a) Enzyme affinity Km of ß-glucosidase (BG), b) chitinase (NAG), and c) leucine-aminopeptidase (LAP) in unplanted, planted and inoculated treatments (n = 4) with applications of WC = wood chips, WS = wheat straw, and WC+WS = wood chips and wheat straw mulch. Different letters indicate significant differences according to ANOVA, 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

The highest catalytic efficiencies were observed for BG, decreasing from NAG to LAP ($p < 0.05$; Figure B 5), whereas K_a of BG was approximately two times higher than that of NAG and almost four times higher than LAP, while K_a of NAG was two times higher than LAP. Under water optimum conditions, mulch affected K_a of BG, and plant and inoculation affected K_a of NAG and LAP (B7 Supplementary Material 5, B7 Supplementary Material 4). Under water stress conditions, K_a of BG was influenced by plant and inoculation, while K_a of NAG and LAP were affected by mulch. In addition, LAP was affected by plant and inoculation and the interaction with mulch (B7 Supplementary Material 5).

Under water optimum conditions, mulch increased K_a of BG ($p<0.05$; B7 Supplementary Material 5). Water stress reduced K_a of BG only in the unplanted WS (-53%) and planted treatment without mulch (-31%; Figure B 5a). For K_a of NAG under water optimum conditions, an increase in the unplanted WS treatment compared to the planted unmulched, planted WC and planted WC+WS treatments was observed ($p<0.05$; Figure B 5b). For LAP, mulch increased K_a only under water stress conditions, for the planted WS treatment ($p<0.05$; Figure B 5c).

Overall, mulch increased K_a of BG under water optimum conditions and K_a of LAP under water stress conditions (B7 Supplementary Material 5).

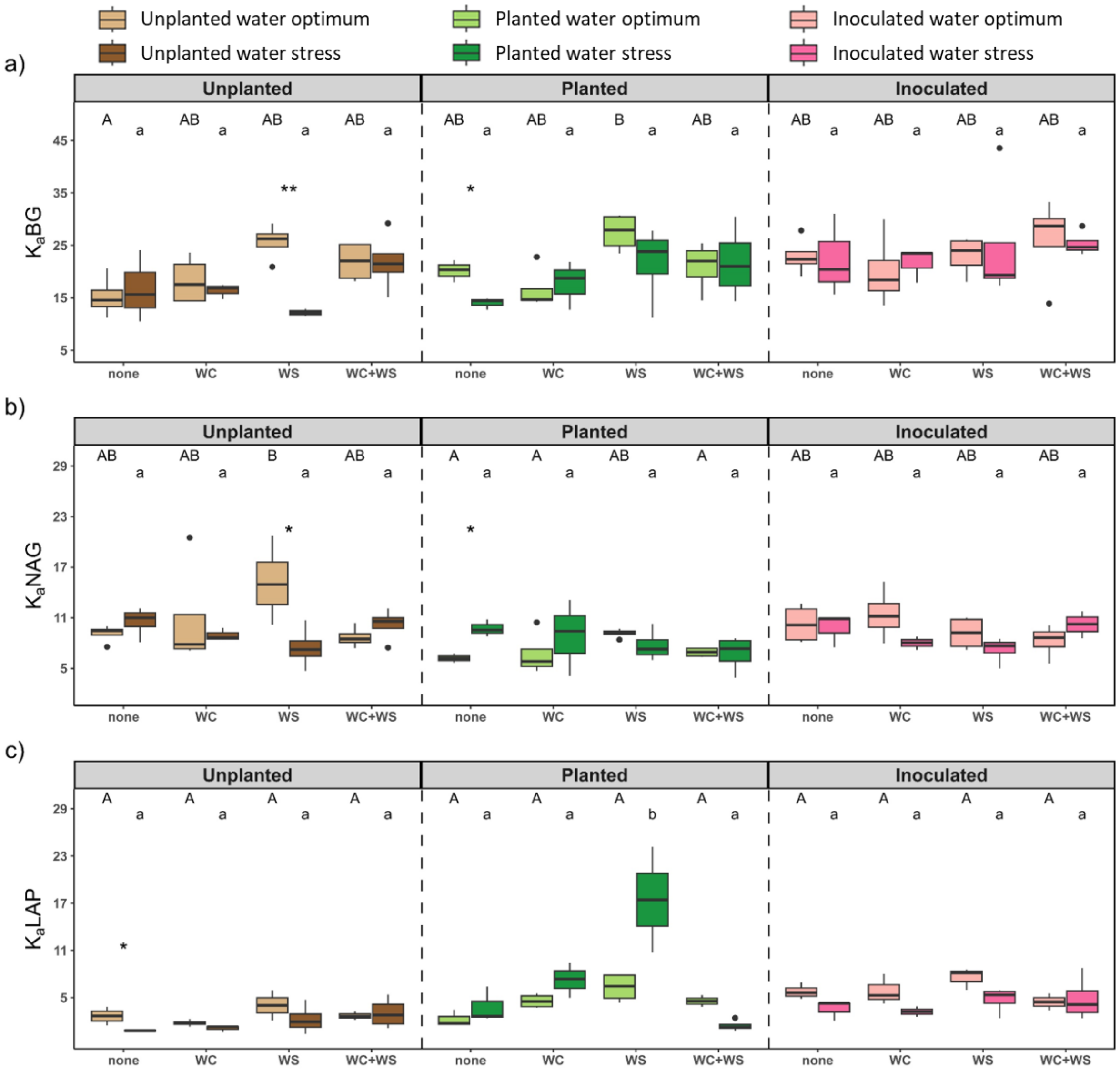

Figure B 5: a) Catalytic efficiency Ka of ß-glucosidase (BG), b) chitinase (NAG), and c) leucine-aminopeptidase (LAP) in unplanted, planted and inoculated treatments (n = 4) with applications of WC = wood chips, WS = wheat straw, and WC+WS = wood chips and wheat straw mulch. Different letters indicate significant differences according to ANOVA, 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

Soil enzymatic C:N ratios revealed that under water optimum conditions, unplanted (WC, WC+WS) and planted treatments (no mulch, WC+WS) were N limited (Figure B 6). In contrast, inoculated treatments (no mulch, WC) were C limited. Under water stress conditions, the C:N balance shifted, and all treatments were N-limited.

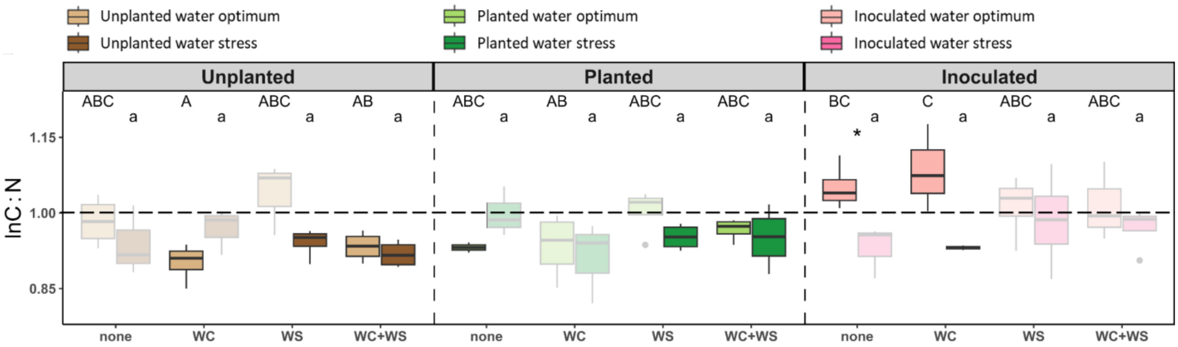

Figure B 6: Enzymatic ratios of C:N (lnBG:ln(NAG+LAP)) in unplanted, planted and inoculated treatments (n = 4) with applications of WC = wood chips, WS = wheat straw, and WC+WS = wood chips and wheat straw mulch. Different letters indicate significant differences according to ANOVA, where capital letters 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 and unshaded boxplots indicate significant difference from 1 according to t-test

B4 Discussion

B4.1 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 (Sapkota et al. 2015; Noor ul et al. 2022; Ortega et al. 2023; Rossi et al. 2024). 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 WS and WC 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 WC and WS 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, WC mulch may still be important to protect against wind erosion, rainsplash and surface runoff (Appiah et al. 2023; Amiri Ghanat Saman et al. 2025) in sandy soils under field conditions.

Although mulch was insufficient to alter SOC and TN, it can still influence microbial processes (Akhtar et al. 2018). Our hypothesis that enzyme activities would increase under mulch application was only partially supported. Mulch alone did not increase BG activities in unplanted and planted soils, but likely supplied labile substrates that supported microbial growth and nutrient turnover (Du et al. 2022; Noor ul et al. 2022; Wang et al. 2025b). In planted unmulched soils, higher LAP activity and substrate affinity compared to planted mulched and inoculated soils likely indicate microbial adaptation to increased soil temperature and substrate scarcity, leading to increased enzyme production rather than enzyme efficiency (Allison and Vitousek 2005; Stone et al. 2012; Razavi et al. 2016a; Stock et al. 2019; Uwituze et al. 2023; Li et al. 2024). Thus, unmulched treatments likely reflected a different soil microclimate. Since enzyme activity (V_{max}) is more temperature sensitive than substrate affinity (K_m) (Razavi et al. 2016a), this likely explains the responses in LAP enzyme activity and substrate affinity in unmulched soils. Further, enzymatic C:N ratios in unplanted and planted soils with mulch (WC, WC+WS) indicated higher BG activity relative to N-related enzyme activity, suggesting that short-term organic inputs preferentially stimulate microbial C rather than N turnover (Noor ul et al. 2022; Uwituze et al. 2023). Although organic mulches often stimulate microbial metabolism and enzyme efficiency (Noor ul et al. 2022; Ortega et al. 2023; Jiang et al. 2025; 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.

B4.2 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). 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 supports 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 supports that legume inputs enhanced microbial N availability (Siczek et al. 2018; Kanté et al. 2023; Liu et al. 2025) 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.

B4.3 Combined effects of mulch application and legume inoculation

As mulch affects 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 mulched WC soils, while other mulched or planted soils showed no effect. This likely reflects both the higher mulch quality of WC, indicated by its lower C:N ratio (Kader et al. 2017; Rossi et al. 2024) compared to WS mulch in our study and the increased N availability through inoculation. Accordingly, higher BG activity in inoculated WC soils likely demonstrates microbial responses to combined availability of labile C and N, as TN can stimulate BG activity (Allison and Vitousek 2005; Stock et al. 2019; Jiang et al. 2025).

B4.4 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 (WC, WS) 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 OC turnover resulting in C stabilisation (Liang 2020; Deng et al. 2021; Qu et al. 2023; Srivastava et al. 2025; Wang et al. 2025b). While TN was unaffected by mulching, individual mulch types in uninoculated soil (WC, WS) 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 (Thilakarathna et al. 2016; Leitner et al. 2017; Zhu et al. 2018; Deng et al. 2021). Moreover, higher TN under water stress conditions in inoculated unmulched and combined mulched soils may indicate increased N inputs from symbiotic N2 fixation, which makes inoculated plants less dependent on the water-driven mass flow of N to the root than uninoculated plants. This aligns with studies demonstrating that inoculation can enhance soil N under drought and improve plant drought tolerance through maintained photosynthetic activity (Jabborova et al. 2021; Álvarez-Aragón et al. 2023; Wang et al. 2025a).

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 (Deng et al. 2021; Bogati and Walczak 2022; 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 dominates microbial nutrient dynamics (Qu et al. 2023; Wang et al. 2025b).

B5 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.

Authors' contributions

Elisa Karina Albrecht: Writing – original draft preparation, Investigation, Conceptualisation, Formal analysis and investigation. Simran Sekhri: Investigation, Methodology, Writing - review & editing. Barbara Reinhold-Hurek: Writing - review & editing, Funding acquisition. Shanmugam Solaiyappan Mani: Writing - review & editing. Svenja C. Stock: Methodology, Writing - review & editing. Michaela A. Dippold: Writing - review & editing, Resources. Annette Eschenbach: Writing - Review & Editing, Supervision, Resources, Conceptualisation, Funding acquisition. Joscha N. Becker: Writing - review & editing, Conceptualisation, Methodology, Supervision.

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B7 Supplementary Material

B7 Supplementary Material 1: Chemical composition of used mulch materials. Carbon (C) and Nitrogen (N) content in percent as well as C:N ratio.

Mulch material	C (%)	N (%)	C:N ratio
Wood chips	44.13	0.72	61.30
Wheat straw	43.14	0.44	97.17

B7 Supplementary Material 2: ANOVA (analysis of variance) results of soil chemical and microbiological properties. SOC= soil organic carbon, TN= total nitrogen, C:N= ratio of SOC and TN, Cmic= microbial biomass

Soil chemical and microbiological properties					
water optimum		term	df	F value	p.value
	SOC	plant_inoc	2	7.2620	< 0.05
		mulch	3	1.6280	0.2006
		plant_inoc:mulch	6	0.6810	0.6656
	TN	plant_inoc	2	2.431	≤ 0.10
		mulch	3	2.687	≤ 0.10
		plant_inoc:mulch	6	1.221	0.3193
	C:N	plant_inoc	2	8.076	< 0.05
		mulch	3	1.812	0.16304
		plant_inoc:mulch	6	4.819	< 0.05
	C_{mic}	plant_inoc	2	1.125	0.33609
		mulch	3	3.824	< 0.05
		plant_inoc:mulch	6	3.968	< 0.05
water stress					
	SOC	plant_inoc	2	1.131	0.3358
		mulch	3	3.604	< 0.05
		plant_inoc:mulch	6	2.262	< 0.05
	TN	plant_inoc	2	19.091	< 0.05
		mulch	3	1.272	0.301
		plant_inoc:mulch	6	1.355	0.264
	C:N	plant_inoc	2	19.385	< 0.05
		mulch	3	0.16	0.9226
		plant_inoc:mulch	6	2.862	< 0.05
	C_{mic}	plant_inoc	2	8.59	< 0.05
		mulch	3	1.229	0.31565
		plant_inoc:mulch	6	3.895	< 0.05

B7 Supplementary Material 3: ANOVA (analysis of variance) results of Vmax (maximal rate of enzymatic activity). BG= β-glucosidase, NAG= chitinase, LAP= leucine-aminopeptidase.

V_{max}	water optimum		treatment	df	F value	p.value
		BG	plant_inoc	2	14.93218033	< 0.05
			mulch	3	1.799449628	0.165292371
			plant_inoc:mulch	6	3.383828267	< 0.05
		NAG	plant_inoc	2	1.011297517	0.374426572
			mulch	3	0.552529174	0.649934304
			plant_inoc:mulch	6	1.933491833	0.103561851
		LAP	plant_inoc	2	11.09458919	< 0.05
			mulch	3	4.349611647	< 0.05
			plant_inoc:mulch	6	1.487961805	0.213784814
	water stress					
		BG	plant_inoc	2	2.502694478	≤ 0.10
			Mulch	3	1.09434158	0.366693157
			plant_inoc:mulch	6	1.75928774	0.141610558
		NAG	plant_inoc	2	3.23156474	≤ 0.10
			Mulch	3	0.267431388	0.848350314
			plant_inoc:mulch	6	1.478685969	0.217955148
		LAP	plant_inoc	2	13.98527308	< 0.05
			Mulch	3	2.036302363	0.129923641
			plant_inoc:mulch	6	3.150845161	< 0.05

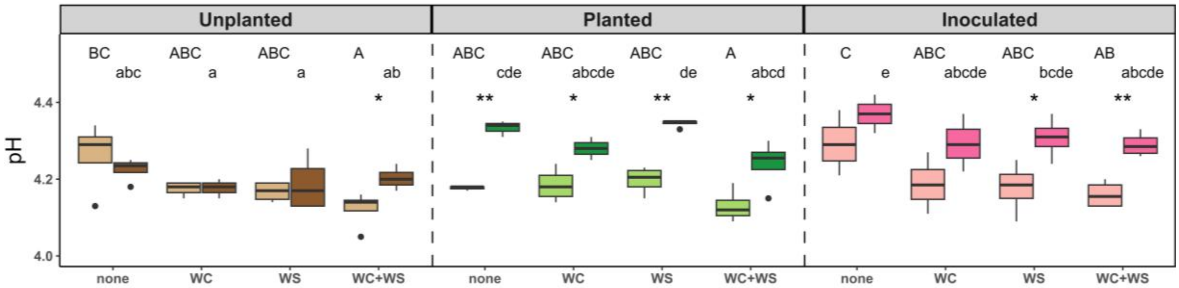
B7 Supplementary Material 4: ANOVA (analysis of variance) results of Km (substrate affinity). BG= β-glucosidase, NAG= chitinase, LAP= leucine-aminopeptidase.

Km	water optimum		Term	df	F value	p.value
		BG	plant_inoc	2	14.93218033	< 0.05
			mulch	3	1.799449628	0.165292371
			plant_inoc:mulch	6	3.383828267	< 0.05
		NAG	plant_inoc	2	1.011297517	0.374426572
			mulch	3	0.552529174	0.649934304
			plant_inoc:mulch	6	1.933491833	0.103561851
		LAP	plant_inoc	2	11.09458919	< 0.05
			mulch	3	4.349611647	< 0.05
			plant_inoc:mulch	6	1.487961805	0.213784814

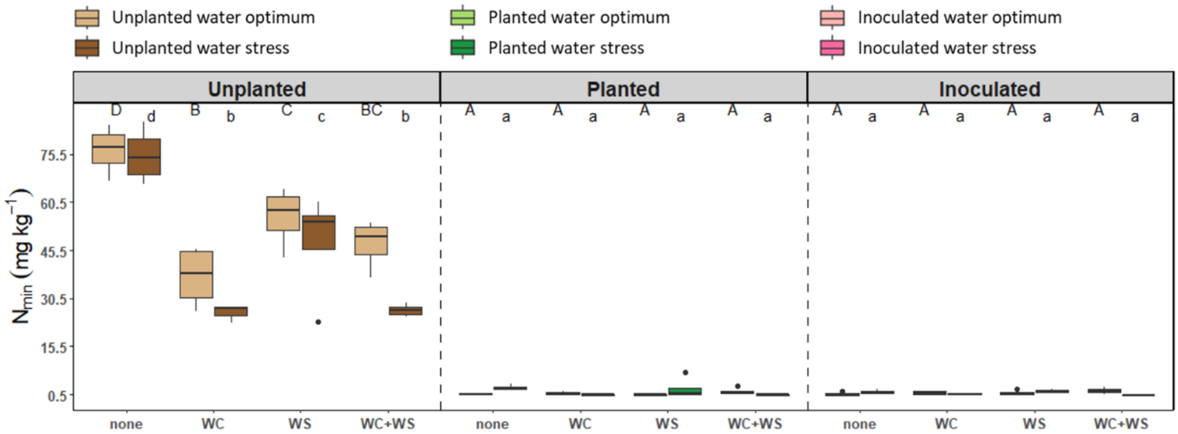
Km	water stress					
		BG	plant_inoc	2	2.502694478	≤ 0.10
			mulch	3	1.09434158	0.366693157
			plant_inoc:mulch	6	1.75928774	0.141610558
		NAG	plant_inoc	2	3.23156474	≤ 0.10
			mulch	3	0.267431388	0.848350314
			plant_inoc:mulch	6	1.478685969	0.217955148
		LAP	plant_inoc	2	13.98527308	< 0.05
			mulch	3	2.036302363	0.129923641
			plant_inoc:mulch	6	3.150845161	< 0.05

B7 Supplementary Material 5: ANOVA (analysis of variance) results of Ka (catalytic efficiency). BG= β-glucosidase, NAG= chitinase, LAP= leucine-aminopeptidase.

Ka	water optimum		term	df	F value	p.value
		BG	plant_inoc	2	1.36750266	0.268017717
			mulch	3	5.536646842	< 0.05
			plant_inoc:mulch	6	1.316268407	0.275905792
		NAG	plant_inoc	2	5.820272082	< 0.05
			mulch	3	2.834825065	≤ 0.10
			plant_inoc:mulch	6	1.522878406	0.200466667
		LAP	plant_inoc	2	7.906067174	< 0.05
			mulch	3	1.247350312	0.312091429
			plant_inoc:mulch	6	1.141495226	0.365775308
	water stress					
		BG	plant_inoc	2	4.473331333	< 0.05
			mulch	3	1.458157887	0.245729697
			plant_inoc:mulch	6	0.783279055	0.589756749
		NAG	plant_inoc	2	1.229196519	0.306392081
			mulch	3	3.014784155	< 0.05
			plant_inoc:mulch	6	1.042421828	0.417402464
		LAP	plant_inoc	2	8.351406664	< 0.05
			mulch	3	4.974125163	< 0.05
			plant_inoc:mulch	6	7.263537943	< 0.05



B7 Supplementary Material 6: pH measured in CaCl2 in unplanted, planted and inoculated treatments (n = 4 per treatment) with different mulch applications of WC = wood chips, WS= wheat straw, and WC+WS= wood chips and wheat straw. Different letters indicate significant differences according to ANOVA, whereas capital letters show significant difference within water optimum condition and small letters within water stress condition. In addition, stars indicate significant differences within one treatment between different water conditions



B7 Supplementary Material 7: Mineral Nitrogen (Nmin) in unplanted, planted and inoculated treatments (n = 4 per treatment) with different mulch applications of WC = wood chips, WS= wheat straw, and WC+WS= wood chips and wheat straw. Different letters indicate significant differences according to ANOVA, whereas capital letters show significant difference within water optimum condition and small letters within water stress condition. In addition, stars indicate significant differences within one treatment between different water conditions

Study C:

Spatio-temporal distribution of enzyme activities in cowpea rhizosphere – the role of plant growth stages and nodule senescence

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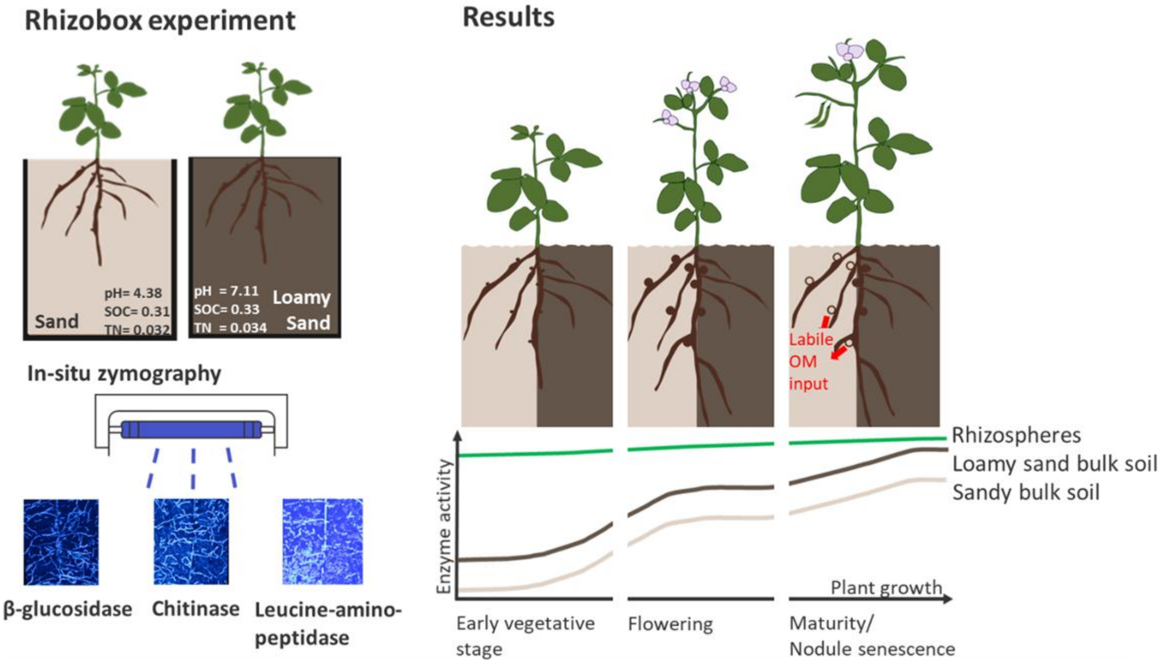
Graphical Abstract



C Abstract

Legume-soil interactions are well recognised for their role in ecosystem nutrient cycling, yet specific mechanisms such as nodule senescence effects on soil nitrogen (N) and carbon (C) cycling remain poorly understood. Here, we investigated the effect of nodule senescence on soil enzyme activity and soil biochemical properties in the rhizospheres of cowpea (Vigna unguiculata) during plant growth. We conducted a rhizobox experiment using soil from the Kavango (loamy sand) and Omusati (sandy soil) regions in Northern Namibia under controlled temperature and optimum water conditions. To investigate spatial and temporal C and N release, in-situ zymography was conducted at early vegetative, flowering, and maturity stage (i.e. one day after the start of nodule senescence) with six replicates per soil. Three enzymes, representing the C (β-glucosidase, chitinase) and N (chitinase, leucine-aminopeptidase) cycle, were investigated. At each plant growth stage, three additional plants per soil were harvested to identify changes in soil properties, including soil organic carbon, total N, mineral N, and pH. Our results showed that enzyme activities did not vary significantly during plant growth in rhizospheres and at nodule and root surfaces. In contrast, enzyme activities significantly increased with plant growth in bulk soil, especially β-glucosidase and chitinase, with a peak at maturity stage. Particularly in the sandy soil, nodule senescence significantly increased enzyme activities. This indicates enhanced organic matter decomposition and nutrient release mainly from the nodule-influenced rhizosphere to the bulk soil and might be attributed to rhizodeposition and microbial responses to substrate availability. We conclude that nodule senescence of legumes is an important driver of enzyme activity and can be a crucial factor for managing soil properties in low-nutrient soils.

Keywords

In-situ Zymography, N and C cycling, nodule senescence, soil-plant interaction, rhizosphere

Highlights

1. Bulk soil enzyme activities increased during plant growth.
2. Rhizospheres, root and nodule enzyme activities stayed stable during plant growth.
3. Senescing nodules released OM, triggering higher enzyme activity.
4. Higher bulk soil enzyme activity in loamy sand than in sandy soil.

C1 Introduction

Legumes are essential for maintaining soil fertility and enhancing soil organic matter (SOM), particularly in low-nutrient soils (Gogoi et al., 2018; Kanté et al., 2021). They are widely used in agricultural systems, in intercropping or crop rotations to provide nitrogen (N) in the soil for following crops (Kanté et al., 2023), while also contributing to soil organic carbon (SOC) through rhizodeposition (Virk et al., 2022). Although legumes are known to especially improve soil N status through biological N fixation in root nodules (Siddique et al., 2012), the dynamics of this process throughout plant growth, particularly during nodule senescence, remain poorly understood. Only a few studies exist on legume-soil interactions during plant growth (Ma et al., 2018a; Siczek et al., 2018), and it is unknown if and how nodule senescence contributes to soil nutrient availability and SOC, particularly in low-nutrient soils.

Legume symbiosis with rhizobia results in the formation of nodules on roots (Yeremko et al., 2025). Through N fixation in nodules, N is released in plant-available forms, such as ammonium, into the soil (George et al., 2016; Tharanath et al., 2024). Like other plant organs, the root nodule has a limited lifespan and enters a senescence process once this lifespan is over. Nodule senescence is characterised by a decline in N_2 fixation and the death of rhizobia and plant cells that potentially enrich the surrounding soil with nutrients (Kazmierczak et al., 2020).

These processes mainly take place in the rhizosphere, which represents a hotspot area between roots and the surrounding soil (Razavi et al., 2016). In this area, rhizodeposition occurs – the release of organic compounds from roots and nodules, which significantly contributes to soil C and N availability (Kanté et al., 2023; Wankhade et al., 2025). These rhizodeposits are rich in labile C, e.g., root exudates, and generate a microbial hotspot for nutrient cycling, and thus stimulate microbial activity (Kuzyakov and Blagodatskaya, 2015; Razavi et al., 2016; Kanté et al., 2023). In addition, root death and nodule senescence enhance the availability of N and SOC through rhizodeposition (Virk et al., 2022). This is particularly relevant in legume cultivation, due to the low C:N ratio of plant tissues, which accelerates root decomposition and nutrient release compared to other crops (Blesh, 2019; Virk et al., 2022).

This decomposition of plant-derived material in soil is driven by extracellular enzymes, which are released by plant roots or microorganisms (Kuzyakov and Razavi, 2019; Liu et al., 2022). Soil extracellular enzymes, such as β-glucosidase, chitinase (N-acetylglucosaminidase), and leucine-aminopeptidase, mediate the decomposition of SOM and the mobilisation of nutrients (Burns et al., 2013; Razavi et al., 2016; Liu et al., 2024). Enzyme activity in the rhizosphere therefore reflects interactions between plants and microbes (Razavi et al., 2016), and higher enzyme activities can be interpreted as greater functional diversity (Gianfreda, 2015). These enzyme-mediated interactions between plants and microbes vary during plant growth due to shifts in physiological processes (Ma et al., 2018a; Siczek et al., 2018; Kanté et al., 2021) and rhizodeposition as well as enzyme activity respond to these variations (Ge et al., 2017; Ma et al., 2018a; Wei et al., 2019; Kanté et al., 2021). For legumes, enzyme activities in rhizosphere and bulk soil have been shown to increase until the reproductive stage (Ma et al., 2018a; Siczek et al., 2018). Later, at maturity stage, increased root decomposition and nodule senescence lead to a temporary release of organic matter (OM) into the soil (Kazmierczak et al., 2020; Kanté et al., 2023). This OM provides labile substrates for microbes, which in turn can stimulate enzyme production and influence nutrient cycling. Consequently, nutrient availability in the rhizosphere is closely linked to rhizodeposition dynamics (Wei et al., 2019) and plant growth stages (Ge et al., 2017; Liu et al., 2017).

However, the response of soil enzymes is governed not only by rhizosphere dynamics but also by soil properties. Differences in soil properties, such as texture and OM content, influence rhizosphere dynamics, including rhizodeposition, and thus microbial and enzyme activity (Islam et al., 2025). In sandy soils with low OM content, microorganisms may respond more sensitively to rhizodeposition, leading to stronger changes in enzyme activity (Wilkerson and Olapade, 2020). However, detailed investigations of enzyme activities in the root and nodule zone, including nodule senescence, across legume growth stages and contrasting soil types are, to our knowledge, still lacking. Here, we investigated the effect of legume cultivation on enzyme activities and soil properties during plant growth and at nodule senescence. To visualise spatial and temporal changes of enzyme activities in the soil and rhizosphere of legumes, *in-situ* zymography was applied at different plant growth stages. This non-destructive technique enables a visualisation of the spatial distribution of enzyme activities in the soil (Razavi et al., 2019). Our study focused on β-glucosidase (C-cycle), chitinase (C- and N-cycle), and leucine-aminopeptidase (N-cycle) due to their key roles in SOM turnover and microbial nutrient acquisition (Chen et al., 2018; Greenfield et al., 2021; Li et al., 2024). We hypothesised that: 1) rhizosphere enzyme activities increase over the course of plant growth, particularly after nodule senescence; 2) this spatio-temporal response is stronger in less fertile soils.

C2 Material and Methods

C2.1 Soil preparation

Topsoil from a depth of 0– 10 cm from two different regions, the Kavango (loamy sand) and Omusati (sand) regions in North Namibia, was used. These regions represent common areas where drought-resistant legumes, such as cowpea and bambara groundnut, are grown to improve soil properties (Horn and Shimelis, 2020; Pasipanodya et al., 2025). After sampling, soil samples were air-dried, homogenised and sieved (< 2 mm). The main soil properties for the loamy sand were pH (1:2.5 $CaCl_2$) 7.11 and 4.38 for the sandy soil, SOC 0.33 % and 0.31 %, total N (TN) 0.0338 % and 0.0315 %, respectively (C7 Supplementary Material 1).

C2.2 Experimental setup

The experiment was designed to simulate one entire plant growth period. Cowpea (Vigna unguiculata), which usually forms determinate nodules that grow through cell enlargement and where nodule senescence starts from the nodule center and extends to the outside (Kazmierczak et al., 2020; Berrabah et al., 2024), was grown under controlled temperature and water availability in rhizoboxes (28x28x1 cm). Seedlings were provided by the Department of Microbe-Plant Interactions of the University of Bremen. Before planting, cowpea seeds (variety Lutembwe) were surface sterilised with freshly prepared 2.5 % sodium hypochlorite for 6 min, washed repeatedly with sterile distilled water and allowed to germinate on 1 % water agar plates for two to 3 day at 30 °C in the dark. This was done to prevent seedlings from possible diseases. To avoid phosphorus deficiency, plants were fertilised with 100 kg ha^{-1} granulated superphosphate, with 18 % phosphorus pentoxide added to each rhizobox soil. Six replicates of each soil were treated to a near-constant water availability of 45 % maximum water holding capacity by weighing and watering each box every 48 hours or the day before zymography. Plants were grown until maturity stage (or until nodule senescence appeared). Nodule senescence was identified by daily visual inspection after flowering stage. Initial senescence was determined based on a colour change from light brown (active nodules) to dark brown or black. Typically, one day after the nodules turned black, complete decay of nodule was observed. Representative photographs of active and senescent nodules are provided in the (C7 Supplementary Material 2). To identify changes in soil chemical properties, for each soil type, three replicates per soil were planted to be harvested directly after each zymography. The rhizoboxes were kept at an angle of 60° to ensure root growth toward the transparent acrylic glass. Plants were grown at 22 °C and relative humidity of 50 % under artificial illumination (six 200µmol/s LED grow lights (Cokolila, GL-CR600)) with a photoperiod of 12 h per day.

C2.3 Soil zymography and imaging processing

At early vegetative, flowering, and maturity stage (or one day after nodule senescence appeared), *in-situ* zymography was conducted to visualise the distribution of extracellular enzymes on the soil surface, around roots and nodules over time. We followed the protocol proposed by Razavi et al. (2016). This involved visualisation of enzyme activities using membranes saturated with 4-methylumbelliferone (MUF)-substrates and 7-amino-4-methylcoumarin (AMC)-substrates. These substrates become fluorescent when enzymatically hydrolysed by the respective enzyme. 4-methylumbelliferyl-β-glucopyranoside was used as substrate to detect β-glucosidase activity; 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide was used as substrate to detect chitinase activity; and L-Leucine-7-amido-4-methylcoumarin hydrochloride for leucine-aminopeptidase activity. Each of these substrates was first dissolved to a concentration of 11mM in DMSO (for MUF substrates) and afterwards in buffer, MES (C6H13NO4SNa0.5) buffer (pH = 6.12±0.13) for MUF substrate, and sterile distilled water for AMC substrate (Sigma-Aldrich, Germany) in autoclaved vessels. InvitrogenTM Nylon membranes (Thermo Fisher, Germany) with a size of 8 x 7.3 cm and a pore size of 0.45 µm were saturated with the substrates for each enzyme. On the day of measurement, rhizoboxes were carefully opened from the lower, rooted site, and the saturated membranes were applied directly to the soil surface and covered with tinfoil and the rhizobox plate to ensure contact between soil and membrane. To ensure that the membrane was placed in the same position for each time step of measurement, the region of interest was marked on the rhizobox. We chose the location corresponding to the initial nodule formation. After an incubation of 1 h, the membranes were carefully lifted off the soil surface, and any attached soil particles were gently removed using a soft brush. Afterwards, the membranes were placed under ultraviolet (UV) illumination with an excitation wavelength of 365 nm and an emission wavelength of 460 nm, in a light-proof chamber (Dark Hood DH-50, biostep GmbH). To maintain constant conditions for all samples, the distance between the UV light source, the camera (Canon EOS 700D, lens: Canon EF 28 mm) and the samples was fixed.

To quantify the zymogram images, a standard calibration that related the activities of various enzymes to the grey value of zymogram fluorescence (i.e. of the saturated membrane) was required. The calibration function was obtained by zymography of 4 cm^2 membranes soaked in a solution with concentrations of 0.01, 0.2, 0.5, 1, 2, 4, 6, 10 mM of MUF and 0, 0.1, 0.25, 0.5, 1, 2.5, 5 mM of AMC. The amount of MUF and AMC on an area basis was calculated from the solution volume taken up by the membrane and its size. The membranes used for calibration were photographed and analysed under the same conditions and in the same way as the zymograms of the samples.

C2.4 Measurement of soil biochemical properties

After each zymography, three additional cowpea plants were destructively sampled from each rhizobox and soil was carefully collected in rhizosphere and bulk soil for further analyses. Before the rhizoboxes were opened at one side, shoots were cut off. Roots were then carefully taken out to preserve them in one piece. Root adherent soil (‘rhizosphere soil’) was sampled by gently brushing off adhering soil from roots after being slightly shaken by hand. Fresh soil was immediately frozen until further analyses. Prior to analyses soil was sieved and homogenised. After rhizosphere soil was sampled, roots were washed and dried at 60°C for 72 h together with the shoot samples. Shoot and root biomass were measured as dry mass.

For ammonium and nitrate analysis (Nmin) an aliquot of 5 g fresh bulk soil was extracted by shaking for 1 h in 20 ml 0.0125 M $CaCl_2$ solution and filtered with 595 ½ cytiva filter papers. Ammonium was analysed at 655 nm with a photometer (DR 5000 Lange Hach). To determine nitrate content, the same extract (0.0125 M $CaCl_2$) was analysed using high performance liquid chromatography (Agilent Technologies, Santa Clara, CA, USA). Rhizosphere and bulk soil samples were dried at 105 °C. Soil and root samples were milled and analysed for total C and N content using a vario MAX cube (Elementar, Langenselbold, Hesse, Germany). The bulk soil pH was measured with a pH electrode in a suspension with 0.01 $MCaCl_2$ (1:2.5).

C2.5 Image processing and analysis

The image processing and analysis were conducted using the open source software ImageJ (using Java) and MATLAB (Version: R2024a). First, the images were transformed into 16-bit grey images using imageJ. Thereafter, zymograms were corrected for light variation of background and quantified by using the calibration lines for MUF and AMC substrates, respectively, using MATLAB (Razavi et al., 2016; Sanaullah et al., 2016; Razavi et al., 2019). Areas of interest (roots and nodules) were identified visually. If possible, three (same) nodules, roots, and bulk soil areas were selected per plant and image for all plant growth stages. The average bulk soil area ranged from 0.2 to 1 cm², depending on the area without visible root influence. To demonstrate rhizosphere activity, a distance of 0.2 mm axially along the root surface was selected to represent the elongation zone of roots and nodules. Soil with a distance of at least 3 mm from rhizosphere areas was referred to as ‘bulk soil’. This represents soil with no direct root influence; however, limited or indirect interactions with the rhizosphere may still occur (C7 Supplementary Material 3).

C2.6 Statistical analyses

For statistical analyses, RStudio (Version 4.5.0) was used. All data are presented as the means of replicates for each treatment ± standard error. Statistical differences were investigated using a linear mixed effect model (LME), with rhizobox ID as random factor and soil type, different segments (nodule, nodule rhizosphere, root, root rhizosphere and bulk soil) and plant growth stage (early vegetative, flowering and maturity) as fixed factors. Assumptions of normality and homogeneity of variance were verified through visual inspection of the model residuals. If LME showed significant differences between treatments, TukeyHSD post-hoc comparisons were conducted. Statistical differences were accepted as significant at p-levels of < 0.05.

C3 Results

C3.1 Spatial distribution of enzyme activities

The activities of β-glucosidase and chitinase exhibited distinct spatial patterns, with highest activities localised around the roots and nodules at the early vegetative stage in both soils (Figure C 1). As plants developed, enzyme activities became more evenly distributed across zymograms in both soils. In the loamy sand, enzyme activity in the bulk soil increased with plant growth stage within the zymogram. The same trend was observed for chitinase activity. In contrast, enzyme activities in the sandy soil remained location-specific around roots and nodules throughout plant growth stage. With plant growth, root and nodule growth increased through the whole rhizobox. Notably, nodule senescence at maturity stage only occurred in the sandy soil.

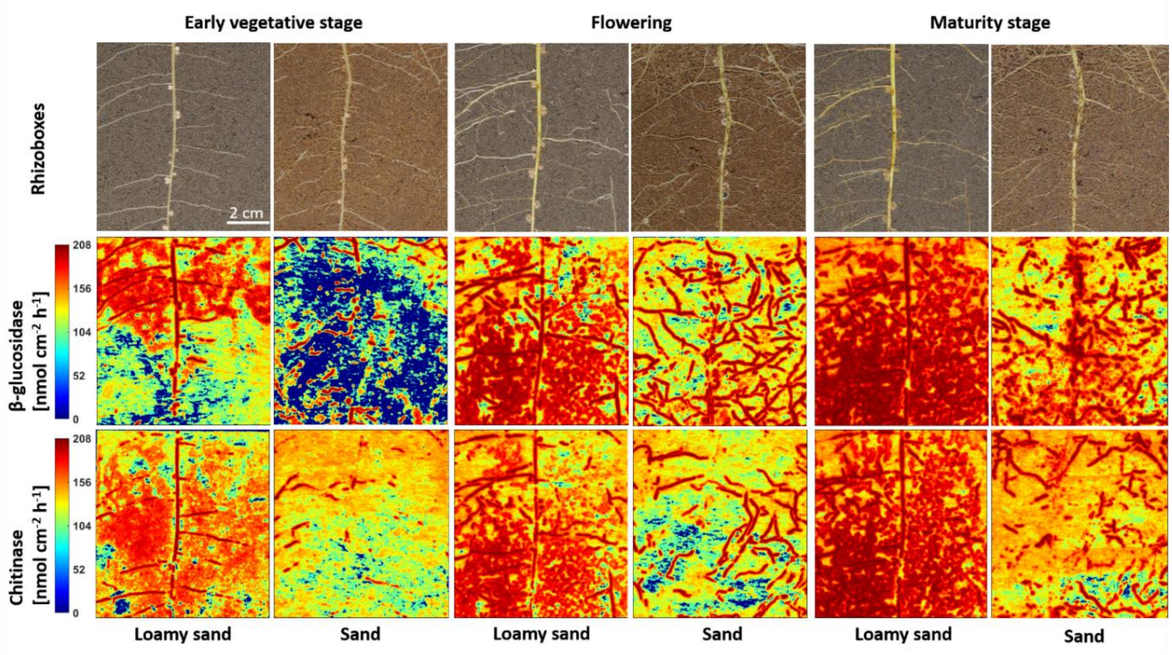


Figure C 1: Spatial distribution of β-glucosidase and chitinase activity (lower rows) with example of cowpea roots grown in rhizoboxes (upper row) at different plant growth stages (early vegetative stage, flowering, maturity stage).

C3.2 Spatiotemporal distribution of enzyme activities

All enzyme activities in bulk soil significantly increased during plant growth in both soils ($p < 0.05$; Figure C 2). Across all plant growth stages, bulk soil enzyme activities were consistently higher in the loamy sand than in the sandy soil ($p < 0.05$). However, bulk soil enzyme activities remained significantly lower than those at root and nodule surfaces and in the rhizospheres. In contrast, enzyme activities at root and nodule surfaces and in the rhizosphere remained stable during plant growth and did not differ significantly between soils.

β-glucosidase activity on root and nodule surfaces as well as in their rhizospheres remained stable during plant growth and did not differ significantly between soils (Figure C 2a). Compared to the bulk soil, β-glucosidase activity in the rhizospheres and at root surfaces was over 100 % higher at the early vegetative stage in the sandy soil, decreasing to 41 % at maturity stage. In the loamy sand, the difference was 45 % at the early vegetative stage and declined to 11 % by maturity stage. Bulk soil β-glucosidase activity in the loamy sand was 44 % higher than in the sandy soil at the early vegetative stage; as activity in the sandy soil increased over time, the difference narrowed to 30 % at maturity stage.

Chitinase activity in bulk soil significantly increased with plant growth in both soils and was consistently higher in the loamy sand, as observed for β-glucosidase activity ($p < 0.05$; Figure C 2b). The extent of soil-specific differences in chitinase activity was comparable to β-glucosidase, decreasing to 25 % at maturity stage. Bulk soil chitinase activity also remained significantly lower than in the rhizospheres of roots and nodules during plant growth (38 % lower at maturity stage; $p < 0.05$). In contrast to β-glucosidase, chitinase activity on nodule surfaces slightly increased during plant development in both soils.

In comparison to chitinase activity, leucine-aminopeptidase activity significantly differed between root and nodule surfaces and their respective rhizospheres. Activity on root and nodule surfaces was significantly higher than in their respective rhizospheres in both soils ($p < 0.05$; Figure C 2c). However, rhizosphere activities of leucine-aminopeptidase showed no significant differences between soils and between plant growth stages.

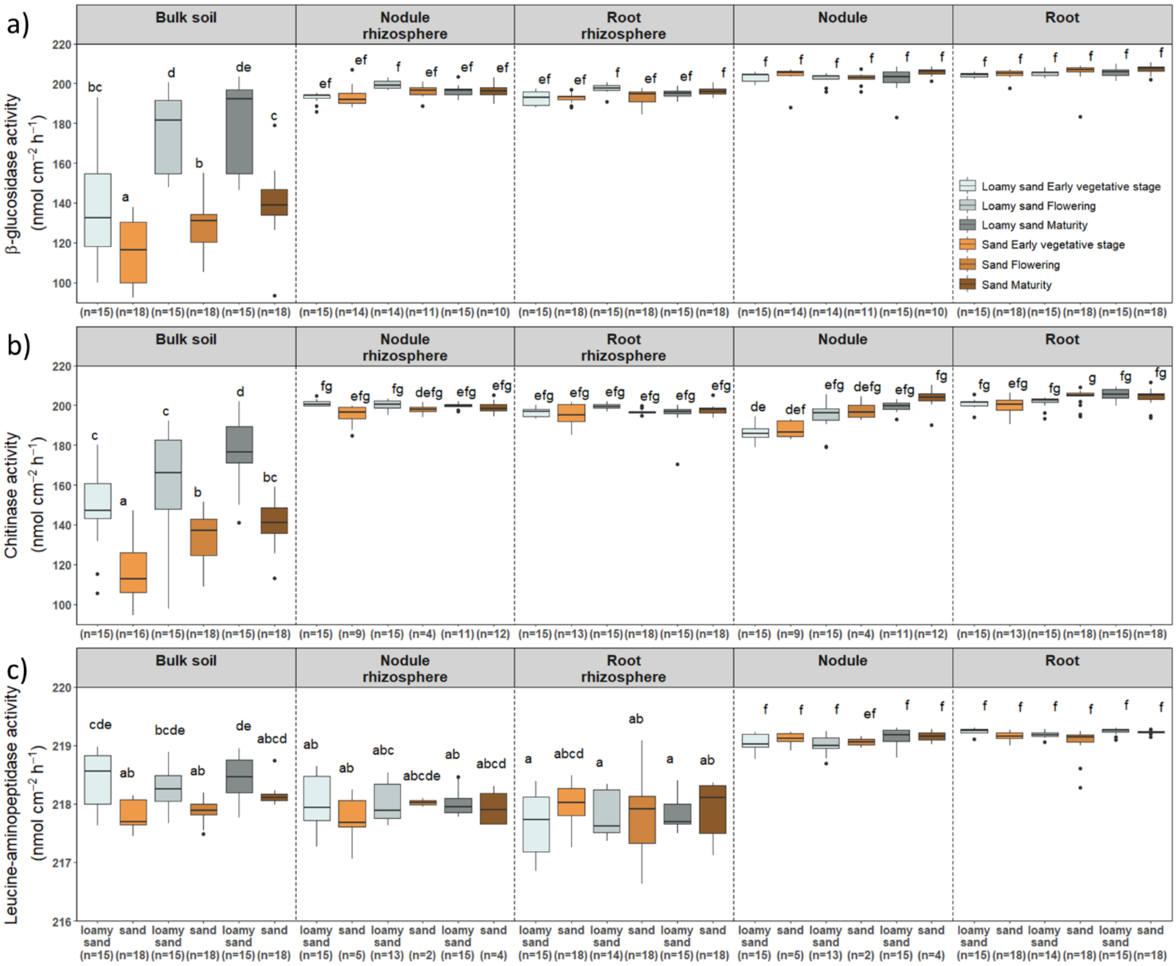


Figure C 2: a) β-glucosidase activity, b) Chitinase activity, c) Leucine-aminopeptidase activity of different plant and soil segments (root, nodule, rhizosphere, and bulk soil) at different plant growth stages. Loamy sand is coloured in grey, and sandy soil in brown, where colour gradients indicate plant growth stages from early vegetative stage (light) to maturity (dark). Sample number (n) represents number of areas of interest (3) per rhizobox (6) per treatment. Lowercase letters indicate significant differences according to linear mixed effect model with Tukey post-hoc test ($p < 0.05$).

C3.3 Effect of nodule senescence on enzyme activities

β-glucosidase activity around the senescent nodules was around 3 % higher compared to root and nodule rhizospheres and significantly higher compared to root rhizosphere ($p < 0.05$; Figure C 3). Additionally, enzyme activity of senescent nodule rhizosphere increased to the same level as nodule surface enzyme activity.

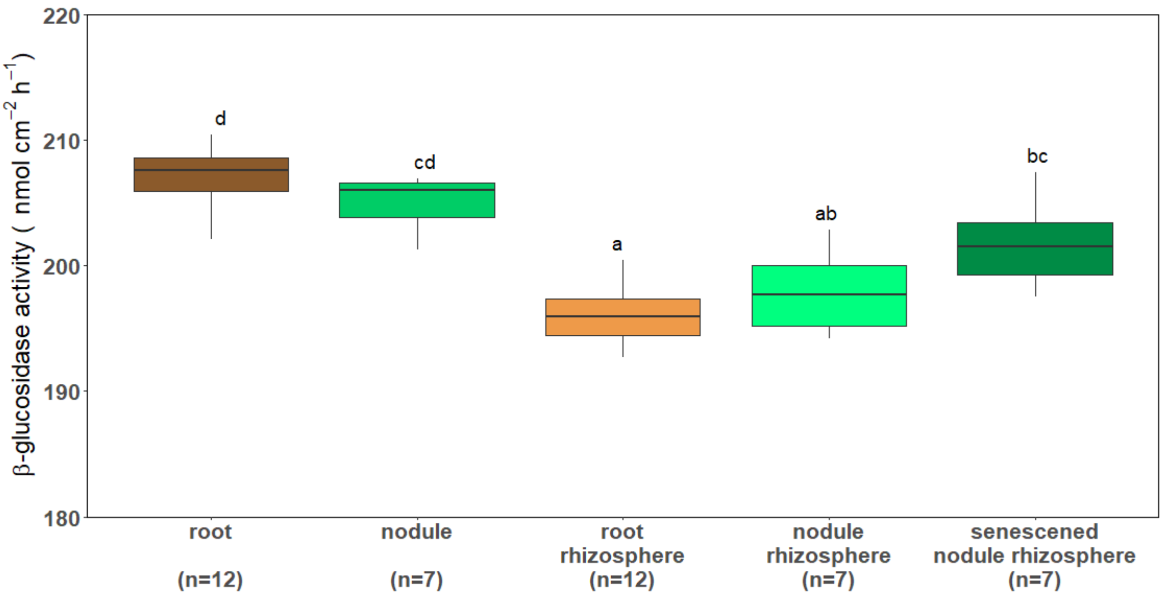


Figure C 3: β-glucosidase activity in sandy soil of different plant and soil segments (root, nodule and respective rhizospheres) at maturity stage in sandy soil. Nodule rhizosphere represents the rhizosphere of other existing nodules within the rhizobox. Sample number (n) represents number of areas of interest (3) per rhizobox (6) per treatment. Lowercase letters indicate significant differences according to linear mixed effect model with Tukey post-hoc test ($p < 0.05$).

C3.4 Plant growth and soil chemical properties

The SOC content was significantly higher in the rhizosphere compared to the bulk soil at flowering and maturity stages ($p < 0.05$; Figure C 4a). Rhizosphere SOC content increased significantly with plant growth in both soils ($p < 0.05$), whereas bulk soil SOC remained stable during plant growth. At maturity stage, rhizosphere SOC in the loamy sand was 32 % and in the sandy soil 23 % higher than in bulk soil ($p < 0.05$).

The TN in both rhizosphere and bulk soils remained within a similar range during plant growth. However, TN concentrations were consistently higher in the loamy sand compared to the sandy soil (Figure C 4b).

In general, the C:N ratio remained stable during plant growth. Until maturity stage, rhizosphere had a higher C:N ratio than the bulk soil (Figure C 4c). By maturity stage, this difference was no longer detectable. Across all growth stages, the C:N ratio in the bulk soil was generally higher in the sandy soil compared to the loamy sand.

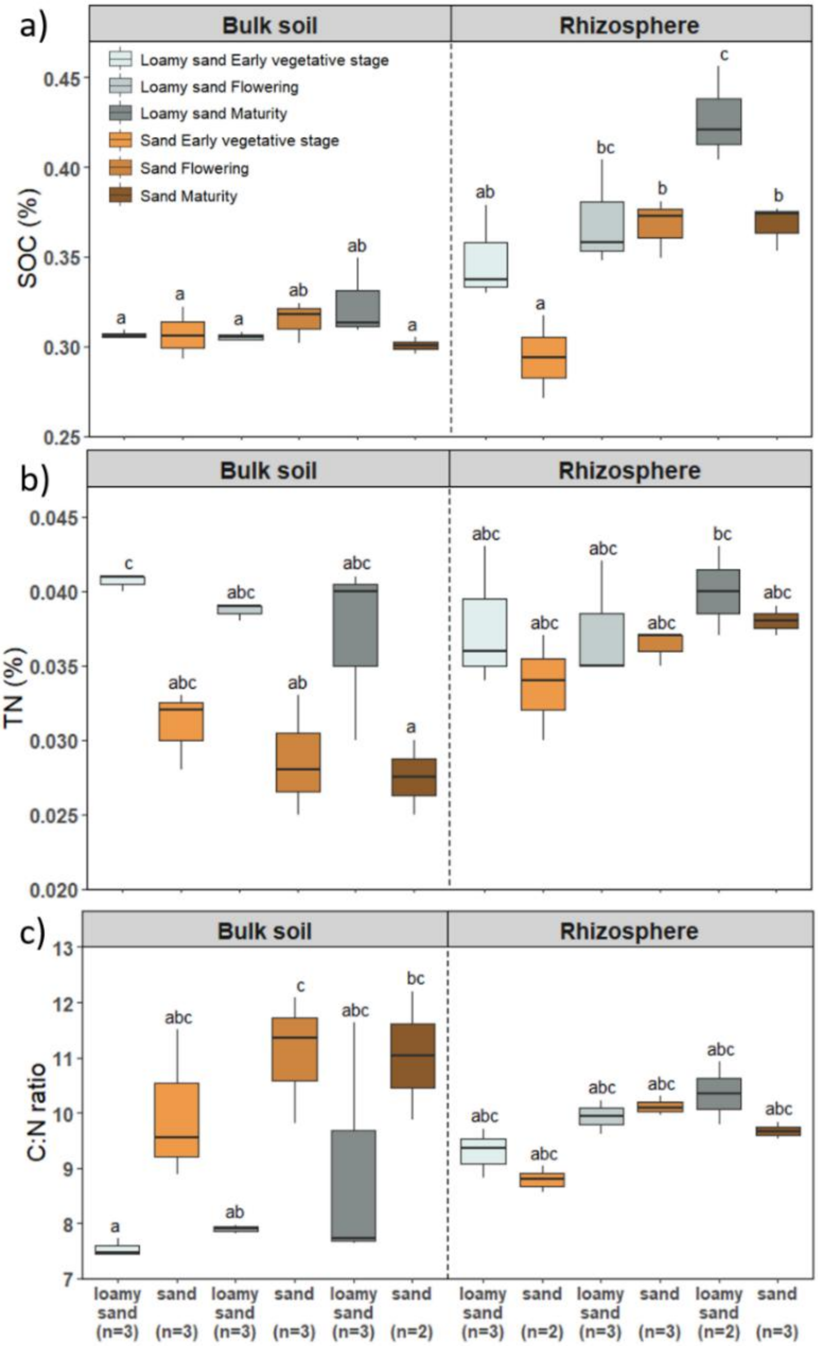


Figure C 4: a) Soil organic carbon (SOC) (in %), b) total N content, and c) the ratio between SOC and TN (C:N) in the rhizosphere and bulk soil at different plant growth stages. Loamy sand is coloured grey, and sandy soil is brown, where colour gradients indicate plant growth stages. Lowercase letters indicate significant differences according to linear mixed effect model with Tukey post-hoc test ($p < 0.05$).

pH values were significantly higher in the loamy sand compared to the sandy soil and remained stable during plant growth in both soils ($p<0.05$; Table C 1). Independent of soil type and plant growth stage N_{min} did not differ significantly (C7 Supplementary Material 4).

Table C 1: pH of bulk soil at different plant growth stages. Values represent means ± standard deviation with n = 3. Letters indicate significant differences ($p < 0.05$).

Soil	Plant growth stage	pH		
Loamy sand	Early vegetative stage	6.53	± 0.24	a
	Flowering	6.77	± 0.05	a
	Maturity	6.76	± 0.14	a
Sand	Early vegetative stage	5.17	± 0.06	b
	Flowering	5.10	± 0.10	b
	Maturity	5.30	± 0.00	b

From early vegetative stage to flowering root biomass increased almost three times ($p<0.05$; Figure C 5a), independent of soil type. The root biomass did not increase from flowering to maturity stage. Root C remained stable until maturity stage in both soils and was significantly higher at flowering compared to early vegetative stage (Figure C 5b). The highest C root content was observed at maturity stage in the sandy soil. Root N remained stable until flowering and declined at maturity stage for both soils, but only significantly for the loamy sand (Figure C 5c). Root C:N ratio remained stable during plant growth (Figure C 5d). At maturity stage, the C:N of roots in the sandy soil was lower compared to the loamy sand. In general, plant growth response in C, N and C:N of roots were stronger in the loamy sand.

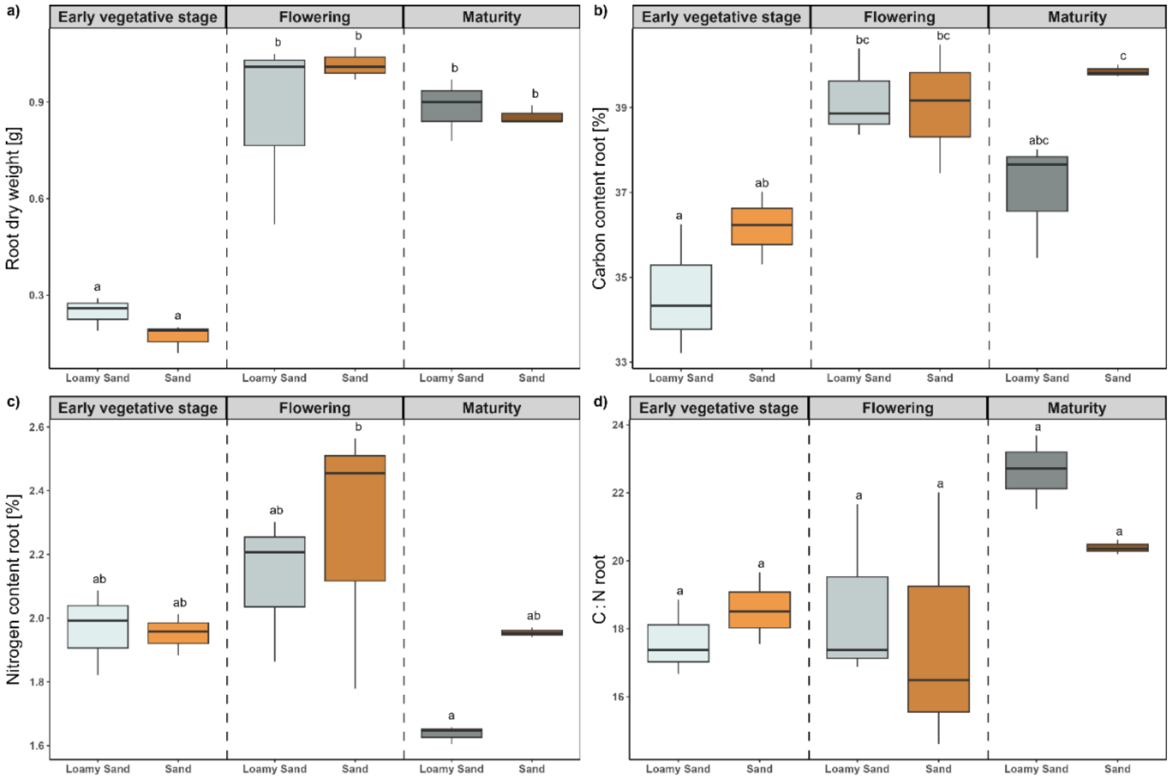


Figure C 5: a) Root dry weight (g), b) carbon content of roots (%) c) nitrogen content of roots (%) d) carbon to nitrogen content of roots at different plant growth stages. Loamy sand is coloured grey, and sandy soil is brown, where colour gradients indicate plant growth stages. Lowercase letters indicate significant differences ($p < 0.05$).

C4 Discussion

C4.1 Spatial and temporal dynamics of enzyme activities

Enzyme activities in the root and nodule rhizospheres, as well as at the root and nodule surfaces, showed clear spatial and temporal variation that was influenced by plant growth stages and soil type. Contrary to our hypothesis, rhizosphere β-glucosidase and chitinase activities remained stable throughout plant growth despite increases in rhizosphere SOC and root biomass. This stability likely reflects continuous input of labile C via root exudation, sustaining microbial activity and enzyme synthesis, or direct enzyme release by roots (Hoang et al., 2016; Ge et al., 2017; Sun et al., 2020; Wankhade et al., 2025). These elevated inputs of labile root exudates combined with stable enzyme activities around roots and nodules, represent a resilient rhizosphere microenvironment (Siczek et al., 2018; Kazmierczak et al., 2020; Wankhade et al., 2025) in our soils cultivated with cowpea. Although temporal stability of enzyme activities has been observed in other studies, enzyme activities observed here (100-220 nmol cm^{-2} h^{-1}) were considerably higher than those reported for lentil and lupine (0.3-13 nmol cm^{-2} h^{-1}) (Liu et al., 2017; Ma et al., 2018a). This suggests that cowpea is particularly effective in enhancing nutrient mobilisation and acquisition in low-nutrient soils, likely sustained by particularly high continuous root exudation compared to other legumes and non-legumes (Dakora and Phillips, 2002; Kanté et al., 2021).

In contrast to the rhizosphere, bulk soil enzyme activities were significantly lower at the early vegetative stage but increased with plant growth. This might reflect an increasing microbial demand for utilisable substrate with ongoing SOM degradation, leading to larger investment in enzyme production (Becker and Holz, 2021; Jat et al., 2021). In particular, the increased β-glucosidase activity in bulk soil at maturity stage suggests enhanced microbial demand for C, resulting from variability of available C compounds that affect microbial activity and overall enzyme production (Allison et al., 2011; Veres et al., 2015; Hoang et al., 2016; Ge et al., 2017; Jat et al., 2021). This aligns with results from Ge et al. (2017), who attributed similar spatiotemporal patterns to the plant growth stage via the effect of root size and root exudation, which is in line with the observed increase in root biomass in our experiment. Although greater nutrient availability can enhance microbial growth and turnover, resulting in higher overall enzyme activity (Hernández and Hobbie, 2010; Sinsabaugh et al., 2014; Hoang et al., 2016; Vinhal-Freitas et al., 2017; Wei et al., 2019), enzyme production is generally induced under nutrient limitation (Allison et al., 2011;

Burns et al., 2013). Thus, nutrient limitation in the bulk soil compared to rhizospheres might have led to increased β-glucosidase activity, reflecting microbial responses to enhance nutrient acquisition through enzyme production (Allison and Vitousek, 2005; Hoang et al., 2016). However, since bulk soil SOC and TN contents remained stable during plant growth, we cannot exclude the possibility that the increase in enzyme activity resulted from labile C compounds or diffusion of enzymes from the rhizosphere (Veres et al., 2015; Wang et al., 2023). While we considered bulk soil as free from visible roots, it still may be affected by the rhizosphere via diffusive processes or microbial interactions (Kuzyakov and Blagodatskaya, 2015; Jat et al., 2021; Wang et al., 2023).

Beyond rhizosphere-mediated effects, enzyme dynamics were also shaped by soil properties. Since root biomass and composition were comparable between both soils, higher enzyme activities in the loamy sand cannot be attributed to root influence alone. Instead, they likely correspond to higher SOC and TN compared to the sandy soil, suggesting that finer texture and greater nutrient status promoted microbial activity, growth and enzyme stabilisation. Consistent with Vinhal-Freitas et al. (2017), who observed higher β-glucosidase activities in clay-rich soils due to SOM protection and enzyme adsorption on clay surfaces (Zimmerman and Ahn, 2010; Zhu et al., 2021; Sheng et al., 2022; Islam et al., 2025). In addition, Sheng et al. (2022) demonstrated that β-glucosidase activity persisted longer when adsorbed onto clay minerals, whereas it decreased faster when associated with primary silicate minerals typical of sandy soils, reflecting differences in adsorption capacity. Higher SOC and TN contents usually stimulate microbial activity and growth (Sinsabaugh et al., 2014; Vinhal-Freitas et al., 2017), which consequently enhances enzyme synthesis and total enzyme activity (Allison and Vitousek, 2005; Wei et al., 2019). This effect could be further enhanced by neutral pH in the loamy sand, which favours microbial activity and enzyme function compared to the more acidic sandy soil (Sinsabaugh et al., 2008). As soil pH remained stable over time, this effect might exacerbate across plant growth.

These observed spatial and temporal patterns of enzyme activities in the rhizosphere of our experiment reflect a dynamic balance between continuous rhizodeposition from cowpea roots and microbial decomposition (Kuzyakov and Blagodatskaya, 2015; Ge et al., 2017), particularly pronounced in the loamy sand.

C4.2 Nodules and N-acquiring enzyme activities

The C- and N-acquiring enzyme (chitinase) slightly increased in nodule enzyme activity with plant growth in both soils. This increase coincided with constant TN in the rhizosphere and increasing root biomass in both soils. Similar patterns have been reported for other legumes, like faba bean, where greater biomass was associated with higher N-acquiring enzyme activities (Kanté et al., 2021), suggesting a balance between N availability and plant growth. This is further amplified by biological N fixation through rhizobia, which release ammonium into the soil (Liu et al., 2025). This ammonium is microbially available and can stimulate microbial growth and enzyme activity (Siczek et al., 2018). Such reduced N demand may shift microbial nutrient mobilisation towards other elements, such as phosphorus (Allison et al., 2011; Tharanath et al., 2024). In legume-based systems, increased enzyme activities thus reflect a combined effect of enhanced N availability (Siczek et al., 2018) and increased legume-rhizobial symbiosis (Virk et al., 2022), especially in N-limited soils (Ma et al., 2018b; Liu et al., 2022).

In contrast to the other measured enzymes, leucine-aminopeptidase showed higher activity in bulk soil than in the rhizospheres. This pattern suggests a high microbial demand for N in the bulk soil, likely due to limited N availability in the studied low-nutrient soils. Such conditions are known to stimulate microbial production of N-acquiring enzymes, including peptidases and chitinases (Egamberdieva et al., 2010; Allison et al., 2011). Legumes influence soil N dynamics not only through biological N fixation (Liu et al., 2022; Liu et al., 2025) but also by releasing N-rich compounds, such as amino acids, into the rhizosphere (Wichern et al., 2008; Kanté et al., 2023). This explains lower enzyme activity in the rhizosphere compared to bulk soil and is a result of higher local N availability derived from legume root exudation, which may reduce microbial demand for N-acquiring enzymes (Wichern et al., 2008).

C4.3 Nodule senescence and enzyme dynamics

Nodule senescence only occurred in the sandy soil and was associated with a pronounced increase in β-glucosidase activity. This supports our first hypothesis that enzyme activities increase after nodule senescence and is also in line with our second hypothesis that the spatio-temporal response is more pronounced in less fertile soils. Enzyme activities in soil surrounding decaying nodules increased significantly compared to the root rhizosphere (Figure C 3). This increase likely reflects a direct input of labile OM from nodule decay, which appeared to outweigh the effect of root exudation. Consistent with findings by Kazmierczak et al. (2020) and Thilakarathna et al. (2016), senescence of roots and nodules of legumes in our study were a significant source of C and N to the soil. This can result in a strong shift in soil C and N turnover due to the low C:N ratio of legume plant parts, compared to other crops (Blesh, 2019; Virk et al., 2022). Such temporarily enhanced inputs of easily available organic compounds are known to fuel fast decomposition by microorganisms (Veres et al., 2015; Blesh, 2019; Sun et al., 2021; Wang et al., 2023) and thus reflect the increased enzyme activities. This observed increase in enzyme activity, especially after nodule senescence in our study, indicates a central role of this process in legume-soil interaction. Nevertheless, further research is needed to assess how this role varies across environmental conditions and to account for differences in nodule type and species-specific plant traits.

C5 Conclusion

We demonstrated that enzyme activities in the rhizosphere and at root and nodule surfaces remain stable during plant growth, likely due to continuous root exudation. In contrast, enzyme activities in the bulk soil followed a different, plant growth-dependent pattern, likely driven by microbial responses to changes in substrate availability. Cowpea cultivation resulted in high enzyme activities during plant growth in low-nutrient soils, which indicates a high potential to mobilise and acquire nutrients under nutrient-limited conditions. Differences in bulk soil enzyme activities between loamy sand and sandy soils suggest that soil properties, along with rhizodeposition in the rhizosphere, play key roles in shaping microbial activity and nutrient mobilisation. Notably, nodule senescence appeared as a key driver of β-glucosidase enzyme activity. Through this temporal input, a significant increase in enzyme activity was identified. Overall, legumes like cowpea can have a key function in maintaining soil fertility and improving SOM in low-nutrient soils. Our results highlight nodule senescence as a previously overlooked spatio-temporal input in the soil. These insights may be important to consider in optimising legume-based cropping systems in low-nutrient soils.

Authors' contributions

Elisa Karina Albrecht: Writing – original draft, Investigation, Conceptualisation, Formal analysis. **Maire Holz:** Writing – Review & Editing, Resources, Methodology. **Joscha N. Becker:** Writing – Review & Editing, Conceptualisation, Methodology, Supervision.

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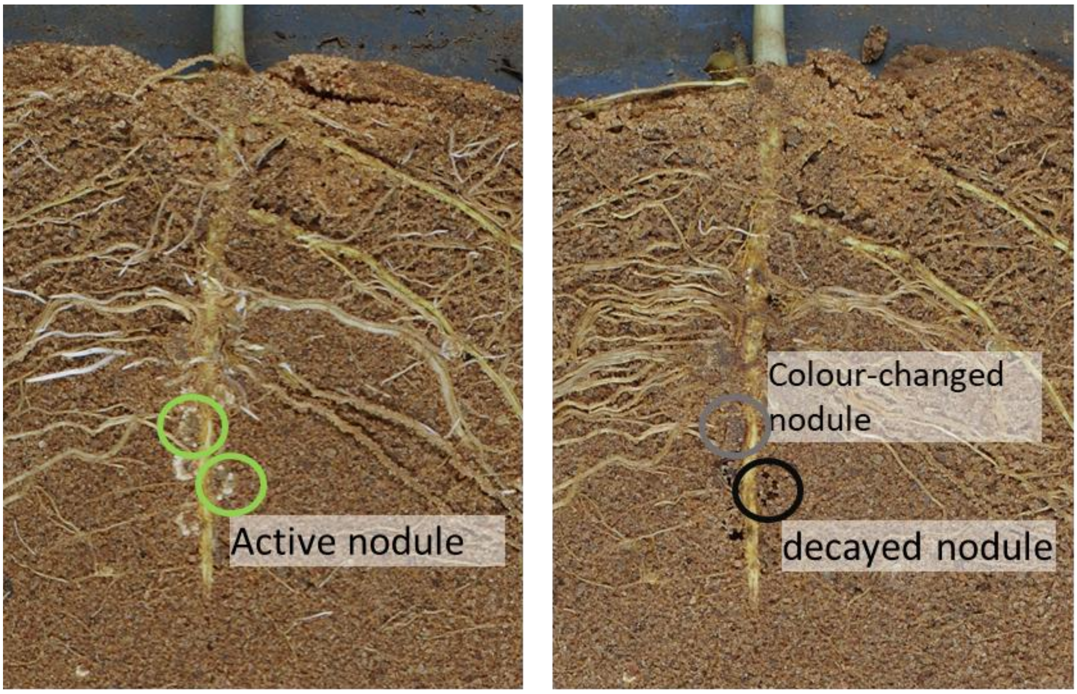
C7 Supplementary Material

C7 Supplementary Material 1: Initial soil properties.

Soil	SOC [%]		N [%]		C/N [%]		pH_{CaCl2}		EC (µS/m)		sand/ silt/ clay [%/%/%]
Loamy sand	0.33	±0.01 a	0.0338	±0.004 a	9.73	±0.91 a	7.11	±0.07 a	77.02	±6.67 a	81/5/15
Sand	0.31	±0.01 b	0.0315	±0.003 a	9.87	±0.70 a	4.38	±0.02 b	12.67	±4.57 b	87/7/6

SOC=soil organic carbon, N=total nitrogen, C/N=carbon to nitrogen ratio, N_{min}= mineral N, pH_{CaCl2}= soil pH in CaCl2, EC=electrical conductivity, BD=bulk density

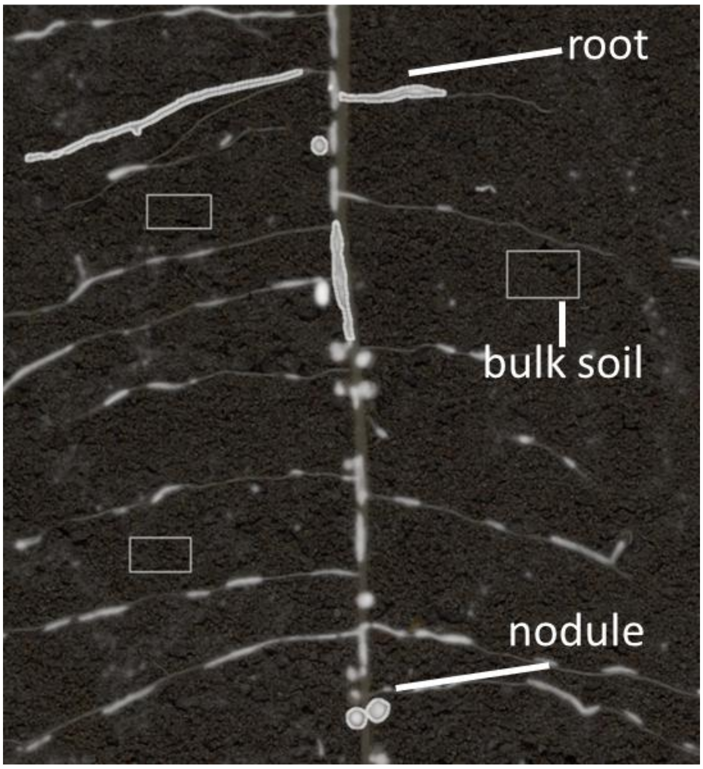
C7 Supplementary Material 2: Assessment of nodule senescence.



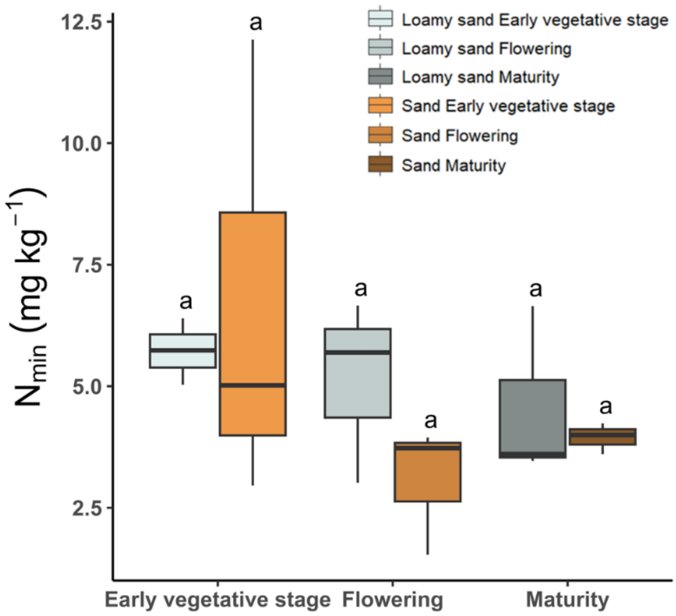
Flowering stage

Maturity stage - nodule senescence

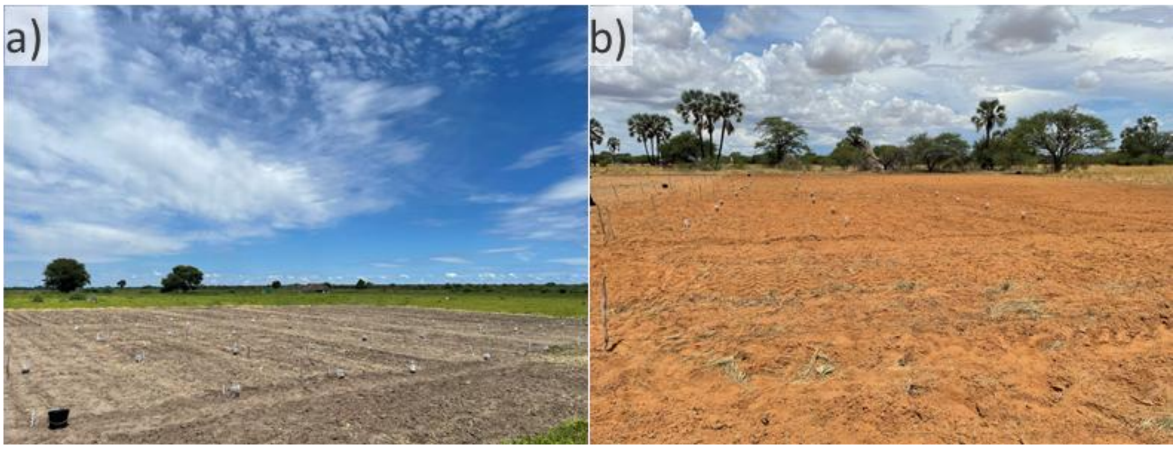
C7 Supplementary Material 3: Example of selected areas of interest. If possible, three nodules, roots and bulk soil areas per rhizobox were selected.



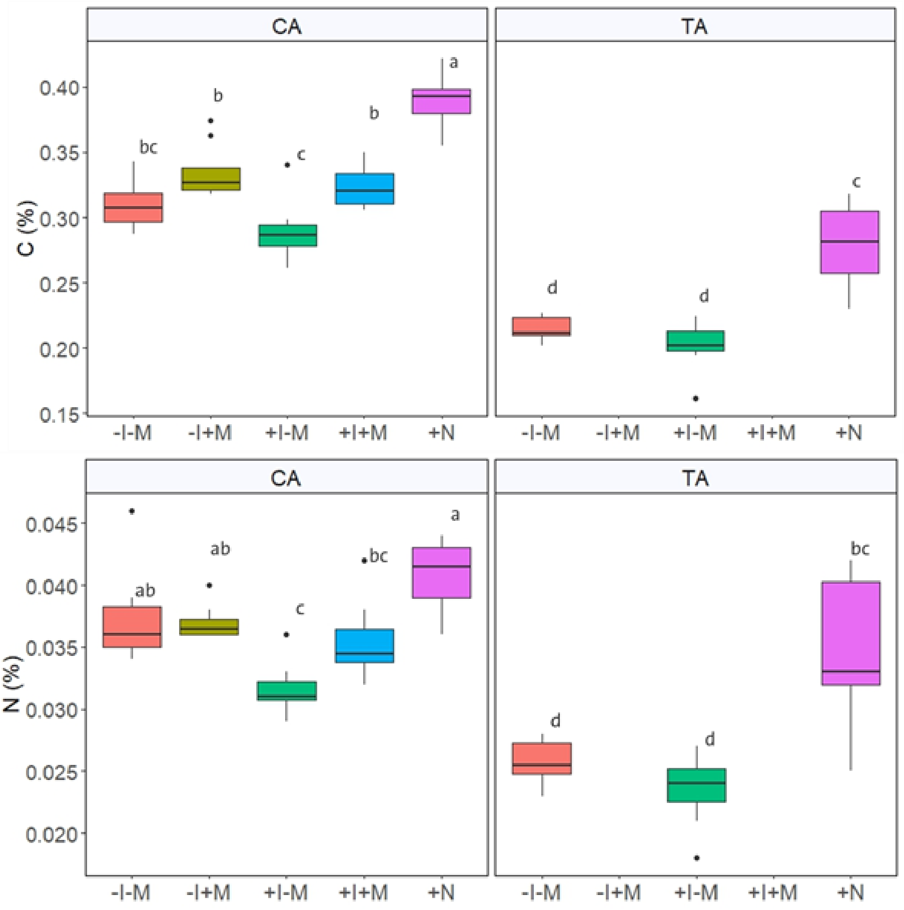
C7 Supplementary Material 4: Mineral Nitrogen (N_{min}) of both soils from the bulk soil during plant growth.



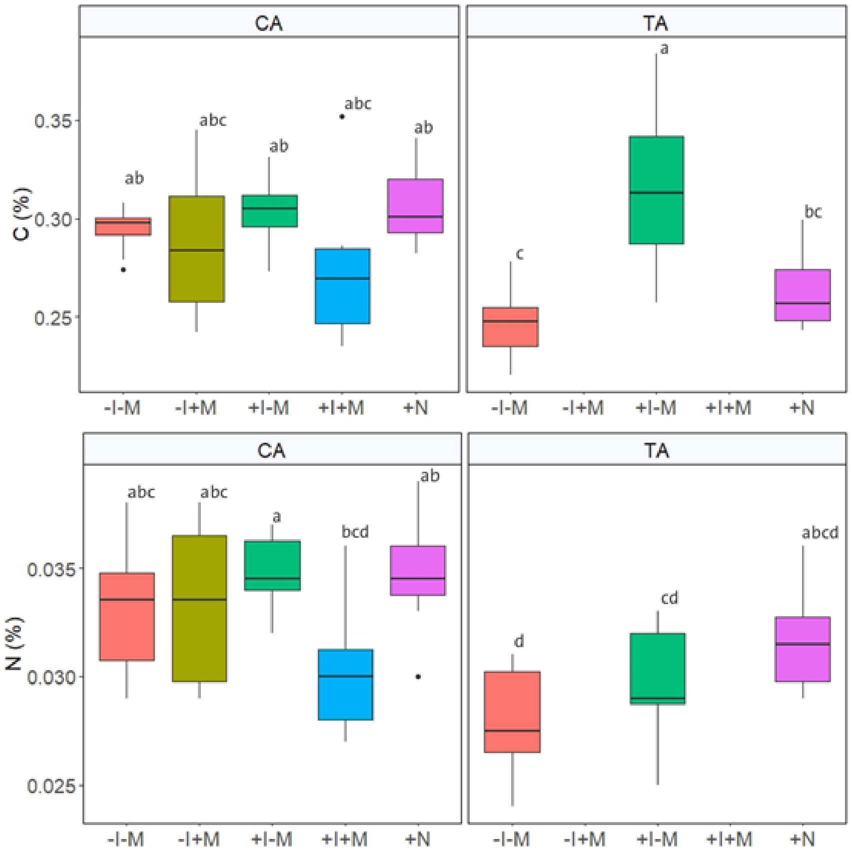
Appendix



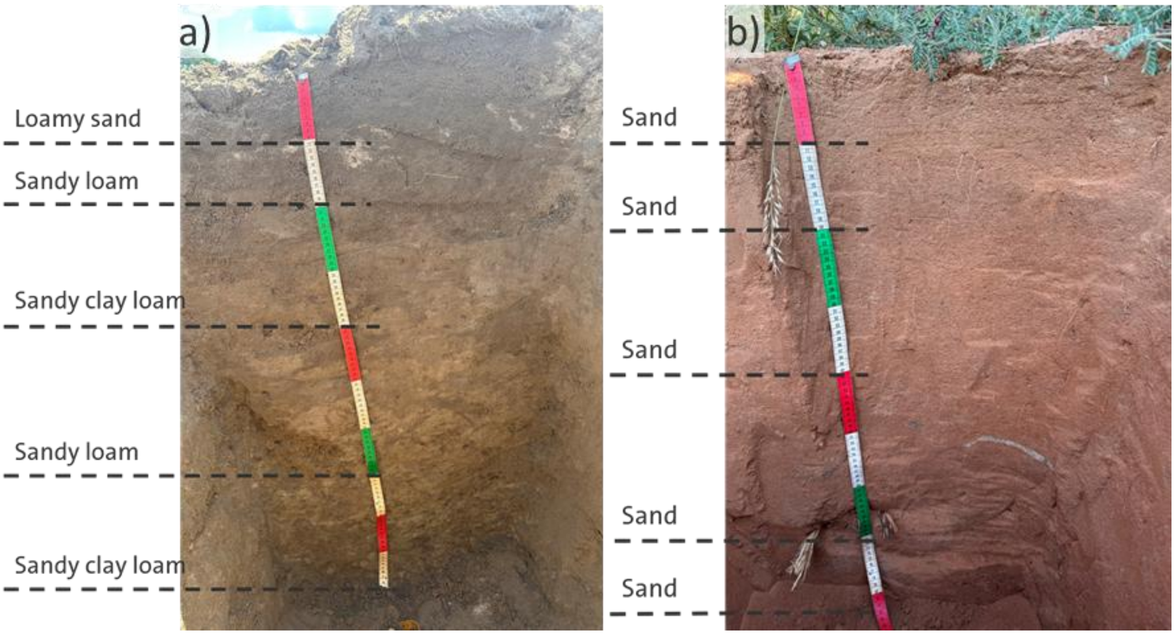
Appendix 1: Established experimental field sites in collaboration with a) the NUST (Namibia University of Science and Technology) in Mashare, located in the Kavango region and b) the UNAM (University of Namibia) in Ogongo located in the Omusati region.



Appendix 2: Initial soil properties of experimental field sites in Mashare. C= carbon, N= nitrogen, I= inoculation, M= mulch, + indicates addition of mulch or inoculant. Letters indicate significant differences.



Appendix 3: Initial soil properties of experimental field sites in Ogongo. C= carbon, N= nitrogen, I= inoculation, M= mulch, + indicates addition of mulch or inoculant. Letters indicate significant differences.



Appendix 4: Soil profiles from the experimental field sites in a) Mashare and b) Ogongo with soil textures in respective horizons.

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