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DIVERSE FORAGE PRODUCTION SYSTEMS AND THEIR POTENTIAL FOR GREENHOUSE GAS MITIGATION

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DIVERSE FORAGE PRODUCTION SYSTEMS AND THEIR POTENTIAL FOR GREENHOUSE GAS MITIGATION

A Thesis
submitted to attain the degree
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This thesis is dedicated to Edith, Elkanah and Hannie Nyameasem.

And God blessed them: and God said unto them, Be fruitful, and multiply, and replenish the earth, and subdue it; and have dominion over the fish of the sea, and over the birds of the heavens, and over every living thing that moveth upon the earth.

Genesis 1:28 (Holy Bible, American Standard Version)

Contents

Abbreviations

AGB	Aboveground biomass
AIC	Akaike information criterion
AIC$_C$	Akaike information criterion, correction for small sample sizes
AMC	7-amino-4-methylcoumarin
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
*a*P	Available phosphorus
ARC	Arable crops
BFN	Biologically fixed nitrogen
BGB	Belowground biomass
BIC	Bayesian information criteria
CFI	comparative fit index
CG	Cut-use grass
CLH	Cut-use legume herbs
CLT	Cut-use legume trees/shrubs
CNLTS	Cut-use non-legume trees/shrubs
CSIR	Council For Scientific and Industrial Research
CTs	Condensed tannins
DM	Dry matter
ECT	Extractable condensed tannins
EF	Emission factor
*e*K	Exchangeable potassium
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization
FCT	Fibre-bound condensed tannins
GHG	Greenhouse gas
GSG	Grazed seeded-grassland
ILCA	International Livestock Centre for Africa
IPCC	Intergovernmental Panel on Climate Change
Irrig	irrigated
IUSS	The International Union of Soil Sciences

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LAP	Leucine aminopeptidase
LCA	Life Cycle Analysis
LTA	Lon-term average
NEE	Net ecosystem exchange
NEP	Net ecosystem production
NGL	Native grassland
NH_3	Ammonia
NI	Nitrification inhibitor
Non-irrig	Non-irrigated
NPP	Net primary production
NUpE	Nitrogen uptake efficiency
NUT	Nitrogen uptake
OM	Organic matter
PCA	Principal Component Analysis
PCT	Protein-bound condensed tannins
PR	Perennial ryegrass
PRN	Patch residual nitrogen
prwc	Perennial ryegrass/white clover swards mixed swards
prwrc	Perennial ryegrass/white clover swards/ Red clover mixed swards
prwrch	Perennial ryegrass/white clover swards/ Red clover/Herbs mixed swards
PTFE	Polytetrafluoroethylene
PVC	Polyvinyl chloride
RC	Red clover
RMSEA	root means square error of approximation
RNUE	Root nitrogen use efficiency
SDG	Sustainable Development Goals
SEM	Structural equation modelling
SNUE	Shoot nitrogen use efficiency
SOC	Soil organic carbon
SOM	Soil organic matter
SRMR	standardized root mean square residual
SSA	Sub-Saharan Africa

*t*N	Total nitrogen
UNCCD	United Nations Convention to Combat Desertification
UPLC	Ultra-Performance Liquid Chromatography
VWC	Volumetric water content
WC	White clover
WFPS	Water-filled pore space
AMF	Arbuscular mycorrhizal fungi

Zusammenfassung

Die Auswirkungen der Landwirtschaft auf die Umwelt sind vermehrt in den Fokus von Politik und gesellschaftlichen Debatten gerückt. Diese Bedeutung wird weiter steigen, da sich der Bedarf an Nahrungsmitteln in den nächsten 2-3 Jahrzehnten verdoppeln soll. Diese negativen Auswirkungen werden in erster Linie der Intensivierung und Vereinfachung der Produktionsysteme angelastet, die sich über sämtliche Produktionsebenen zeigt. Der Grünlandnutzung kommt eine Art Sonderrolle bei, da sie sowohl zum Klimawandel beitragen, aber auch durch besonders hohe Kohlenstoffeinträge als Instrument zur Verringerung der Treibhausgasemissionen eingesetzt werden kann. Hier sind vorallem Low-Input systeme vielversprechend, da diese häufig auch die Biodiversität fördern. Doch sind viele Einflussgrößen auf die Klimabilanz dieser Systeme noch ungewiss, insbesondere da Rückkopplungen zwischen der Pflanze und dem Boden den Kohlenstoff- und Stickstoffkreislauf im Boden über Erntereste, Wurzelzersetzung und Wurzelexsudate steuern. Diese Auswirkungen können jedoch je nach Boden- oder Vegetationstyp variieren. Die Verbesserung der Fähigkeit von Low-Input-Systemen den Klimawandel abzuschwächen erfordert somit eine representative Datengrundlage für viele verschiedene Produktionssysteme. Daher wurde in Kapitel 2 dieser Arbeit der Einfluss einer Reihe von verschiedenen Pflanzenarten im Vergleich zu einem Dauergrünlandsystem auf die C-Einträge und -Austräge einer tropischen Savanne nach mehr als 50 Jahren gleichbleibender Landnutzung bewertet. Es wurden Bodenproben (0-30 cm Tiefe) von beweidetem Grasland, gemähten Dauerkulturen und Dauergrünland genommen. Die Vorräte an organischem Kohlenstoff (SOC) im Boden reichten von 17 bis 64 Mg SOC ha^{-1} (Mittelwert±Sd = 32,9±10,2 Mg ha^{-1}). Die SOC-Vorräte waren bei beweidetem, temporären Grünland niedriger als bei gemähtem Gras, Leguminosen und Sträuchern. Dementsprechend wurde geschätzt, dass bei der Umwandlung von Dauergrünland in Weideland 44 % des SOC über den Zeitraum von 50 Jahren verloren gingen, während die Umwandlung in bestimmte Leguminosen zu geringen (5%) zusätzlichen Gewinnen führte. Innerhalb der Ansaatsysteme schien die Verfügbarkeit von Stickstoff (N) der kritischste Faktor zu sein, der das Schicksal der SOC-Vorräte bestimmt, wobei die N-Konzentration im Boden und der SOC hoch korreliert waren ($r = 0{,}86$; $p<0{,}001$). Insgesamt waren N, P und K signifikante Prädiktoren für die SOC-Dichte in den Böden. Ein Experiment zur Überprüfung der Hypothese, dass leguminosenbasierte N-Quellen durch signifikant niedrigere Emissionsfaktoren gekennzeichnet sind als mineralische N-basierte Milchsysteme, wurde in Kapitel 3 beschrieben. In dieser Studie wurden die N_2O-Emissionen über einen Zeitraum von mindestens 100 Tagen und bis zu zwei Vegetationsperioden über einen Gradienten der Pflanzenartenvielfalt überwacht. Die Emissionen wurden von Weiden und einer kontrollierten Ausbringung von Urin und Dung unter Verwendung der statischen Kammermethode gemessen. Etwa 90 % der kumulierten N_2O-Emissionen der simulierten Experimente traten während der ersten 60-75 Tage auf. Die durchschnittlichen akkumulierten N_2O-Emissionen betrugen 0,11, 0,87, 0,99 und 0,21 kg ha^{-1} für die Kontrolle, sowie die Apllikation von Dung und Urinflecken, sowie der beweideten Weideflächen. Die N-Aufnahmeeffizienz bei den Ausscheidungsflecken lag sowohl für Dung als auch für Urin bei etwa 70 %. Doch selbst der höchste N_2O-N-Emissionsfaktor war um mehr als die Hälfte geringer im Vergleich zum IPCC-Standard (0,3 vs. 0,77), was auf eine Überschätzung der N_2O-N-Emissionen von ökologisch bewirtschafteten Weiden in gemäßigten Klimazonen hindeutet. Die Pflanzendiversität zeigte keinen signifikanten Effekt auf die N_2O-Emission. Daher wurde für ein besseres Verständnis der Mechanismen, mit denen Pflanzen die N_2O-Emission beeinflussen, in Kapitel 4 in dieser Arbeit ein Gewächshausexperiment beschrieben, in dem die Hypothese getestet wurde, dass ein hoher Anteil von Spitzwegerich (*Plantago lanceolata* (PL)) gegenüber dem deutschen Weidelgras (*Lolium perenne* (LP)) in Mischungen die N_2O-Emission reduzieren kann. Der Gewächshausversuch wurde in einem 3x3 faktoriellen, voll randomisierten Design angelegt, das drei mineralische N-Düngerraten (0, 150 und 300 kg N ha^{-1}) umfasste, die auf Monokulturen der beiden Arten und eine binäre Mischung ausgebracht wurden. Gemessen wurden die täglichen N_2O-Emissionen, die oberirdische (AGB) und unterirdische Biomasse (BGB), die N- und C-Erträge ober- und unterirdisch sowie die Leucin-Aminopeptidase (LAP)-Aktivität im Boden als Indikator für die mikrobielle Aktivität im Boden. Die Ergebnisse zeigten, dass die akkumulierten N_2O-Emissionen (83 Tage) bei der PL Monokultur etwa doppelt so hoch waren wie bei der Mischung oder der LP Monokultur ($p<0,05$). Zudem lieferte PL weniger Trockenmasse (AGB+BGB) und N Ertrag als LP oder LP-PL ($p<0,001$). Die binäre Mischung erzielte die höchste N-Nutzungseffizienz und LAP-Aktivität. Die Arbeit zeigt somit generell das Potential von Mischungen die Umweltauswirkungen der Landwirtschaft zu generieren. Trotz der Identifikation einiger Einflussgrößen auf die Nährstoffkreisläufe, und der Erkenntnis, dass Emissionsfaktoren in Grünlandsystemen in gemäßigten Klimaten überschätzt werden, bleiben weiterhin Unsicherheiten bestehen die eine genaue Abschätzung der Emissionen über verschiedene Bodentypen schwierig machen.

Summary

The impact of agriculture on the environment has become a sharp focus, especially as future demand for food is projected to double over the next 2-3 decades. The negative impact of agriculture on the environment is primarily blamed on intensifying and simplifying production evident on the field, farm, landscape or regional scale. Grassland use contributes to climate change and environmental degradation, but it can also be used as a tool for greenhouse gas emissions mitigation. Moreover, low-input systems are often considered ecologically beneficial, but their sustainability under intensification is uncertain. Plant-soil feedbacks regulate soil carbon and nitrogen cycling via litterfall, root decay and root exudates, controlling microbial population dynamics in most soils, yet their impact may vary depending on the vegetation type. Enhancing the capacity of low-input systems to resist soil degradation and mitigate climate change requires long-term assessments. In *Chapter 2*, this thesis assessed the outcome of C inputs and outputs across an array of plant functional groups in arable and permanent systems of a tropical Savannah after more than 50 years of consistent land use. Soil samples were taken (0–30 cm depth) from arable crop fields, grazed-seeded grassland, cut-use permanent crops and native grassland. Soil organic carbon (SOC) stocks ranged from 17 to 64 Mg SOC ha^{-1} (mean±sd = 32.9±10.2 Mg ha^{-1}). SOC stocks were lower for grazed-seeded grassland relative to cut-use grass, legume trees and shrubs. Accordingly, while converting the native grassland to grazed pastures was estimated to have lost 44 % of SOC over the period, the conversion to woody legumes resulted in slight (5 %) incremental gains. Within sown systems, nitrogen (N) availability seemed to be the most critical factor that determines the fate of the SOC stocks, with soil N concentration and SOC being highly correlated ($r = 0.86$; $p<0.001$). In total, N, P and K were significant predictors of SOC density in the soils. Experiments to test the hypothesis that legume-based N sources are characterized by significantly lower emission factors than mineral N based dairy systems were described in *Chapter 3.* This study monitored N_2O emissions for a minimum of 100 days and up to two growing seasons across a gradient of plant species diversity. Emissions were measured from grazed pastures and a controlled urine and dung application using the static chamber method. About 90% of the simulated experiments' accumulated N_2O emissions occurred during the first 60–75 days. The average accumulated N_2O emissions were 0.11, 0.87, 0.99, and 0.21 kg ha^{-1} for control, dung, urine patches, and grazed pastures, respectively. The N uptake efficiency at the excreta patch scale was about 70% for both dung and urine. The highest N_2O-N emission factor was less than half compared with the IPCC default (0.3 vs 0.77), suggesting an overestimation of N_2O-N emissions from organically managed pastures in temperate climates. Plant diversity showed no significant effect on N_2O emission. However, functional groups were significant ($p < 0.05$). Moreover, soil-plant interactions affecting N_2O production in soils are not well understood, and experimental data are scarce. A better understanding of the mechanisms by which plants affect N_2O emission would facilitate the selection of forage species for sustainable intensification. In *Chapter 4*, this thesis described a greenhouse experiment that tested the hypothesis that high forage plantain *Plantago lancelota* (PL) than *Lolium perenne* (LP) in mixtures reduces N_2O emission. The greenhouse experiment was arranged in a 3x3 factorial, fully randomized design, comprising three mineral N fertilizer rates (0, 150 and 300 kg N ha^{-1}) applied to two monocultures (*LP* and *PL*) and one binary (*LP-PL)* sward mixture. Parameters measured included daily N_2O emissions, aboveground (AGB) and belowground biomass (BGB), N- and C- yields above- and belowground, as well as leucine aminopeptidase (LAP) activity in the soil as an indicator for soil microbial activity. Results showed that accumulated N_2O emissions (83 days) were about two times higher for *PL* than *LP* or *LP-PL* swards ($p<0.05$). N application increased AGB in all treatments ($p<0.001$), however, the N300 rate reduced biomass allocation belowground. The *PL* yielded lower dry matter (AGB+BGB) and N than *LP* or *LP-PL* ($p<0.001$). As a result, the binary sward (LP-PL) showed the highest N use efficiency and increased LAP activity. Spearman correlation analyses and structural equation modelling suggested C allocation dynamics belowground as a potential means by which plants might impact N_2O emission. Thus, the work generally demonstrates the potential of mixtures to reduce the environmental impacts of agriculture. Despite identifying some variables influencing nutrient cycling and recognising that emission factors are overestimated in grassland systems in temperate climates, uncertainties remain, making the accurate estimation of emissions across different soil types difficult.

CHAPTER 1

General introduction

1.1 Global agriculture needs to grow sustainably

In 2017, 11% of the world population suffered from hunger, with undernourishment rates increasing since 2014 and being most prevalent in Sub-Saharan Africa (JANSSENS *et al*., 2020). At the same time, about USD 1 trillion worth of eatables is discarded or lost each year due to lack of infrastructure in developing countries or waste in the developed countries (FAO, 2013). Food consumption also results in environmental problems, particularly in the western world, where food production is estimated to account for 20-30% of the negative ecological impacts (REYNOLDS *et al*., 2014). These pressing problems are exacerbating as the world population is increasing with implications for food demand. Already, food demand is projected to grow substantially at 54% by 2050 compared with 2010, as the world population hits 9 billion (TALLIS *et al*., 2018). Correspondingly, due to shifts in diet, meat and dairy demand are expected to rise by even 76% and 65%, respectively, by 2050, compared with 2005 (ALEXANDRATOS & BRUINSMA, 2012). These high increments are driven by urbanization and income growth in developing countries (SATTERTHWAITE *et al*., 2010). As calories from meat and dairy require more energy during production than vegetarian diets (PIMENTEL & PIMENTEL, 2003), the existing overproduction combined with increasing demand for agricultural products and more energy-intensive animal-based products will increase the requirements for sustainable intensification to reduce environmental impacts.

The impact of agriculture on the environment is different for temperate and tropical regions. For instance, whereas soils in many developing countries are hugely degraded and impoverished in nutrients due to inadequate or lack of nutrient replenishment or poor soil management practices, many developed nations are high in soil organic matter (SOM) and nitrogen (N), most times leading to high deposition of reactive N in those ecosystems. In any case, it is imperative that as efforts are made to close yield gaps in the less developed countries, the mistakes of "industrialized countries" are not repeated. This caution is particularly relevant, as previous efforts to intensify agricultural systems have been characterized by a high input of non-renewable resources and simplification of diversity levels across the field, farm landscape or regional scales (HENDRICKSON *et al*., 2008; LEMAIRE *et al*., 2015; RUSSELLE *et al*., 2007). This was particularly the case in industrialized countries but also in less developed countries (LEMAIRE *et al.*, 2015). Over the years, the over-simplification of agricultural systems from crop rotations to monocropping, and diverse mixtures to monocultures, has resulted in the interrelated problem of ecosystem destruction, soil and water body degradation, as well as biodiversity losses (SCHULTZE-KRAFT *et al.*, 2018). These changes were also visible in the livestock sector, a significant source of greenhouse gas emissions worldwide (SMITH *et al.*, 2008). Agricultural activities generate multiple greenhouse gasses, contributing 10%–14% to global anthropogenic GHG emissions (SMITH *et al.*, 2007; TUBIELLO *et al.*, 2013), predominantly from farm machinery and soil cultivation (carbon dioxide), enteric fermentation (methane) and application of synthetic fertilizers and manure (nitrous oxide) (FIELD *et al.*, 2012). These emissions, particularly nitrogen and carbon, shall be looked at in more detail. Nitrous oxide emission from anthropogenic sources, to which agriculture contributes about 60% to global emissions (SYAKILA & KROEZE, 2011), is of primary environmental concern due to its global warming (265–298 times greater than carbon dioxide) and stratospheric ozone depletion (IPCC, 2013; OENEMA *et al.*, 2009; PORTMANN *et al.*, 2012).

1.2 The impact of climate change on agriculture

While agriculture contributes to climate change, it also is affected by it; however, the extent and mode of change will vary regionally. Sub-Saharan Africa (SSA) is considered to be the most vulnerable region to the current and future climate variability with impacts on rainfall (CHRISTENSEN *et al.*, 2007; NIANG *et al.*, 2014) and could reduce agriculture between 20–50% by 2050 (SARR, 2012). For Northern Germany, on the other hand, an initially beneficial extension of the growing season will likely be outweighed by large increments in drought events in summer (TRNKA *et al.*, 2011). Already, the mean global atmospheric CO_2 concentration and temperature has been steadily increasing (EKWURZEL *et al.*, 2017; GOGLIO *et al.,* 2015) with ramifications for crop productivity and livestock performance in Africa and Europe (US EPA, 2016). The increasing incidence of drought in northern Germany has reduced pasture yield (EMADODIN et al., 2021), while crop losses attributable to climate change are evident in West Africa (SULTAN *et al.*, 2019). Moreover, the hitherto agricultural lands are under intense competition from other sectors, making land increasingly less available for agricultural use (VAN ZANTEN *et al.*, 2018). This trend is particularly problematic, as there is a considerable yield gap in many developing countries due to low investment in agriculture and innovation.

Consequently, general improvements in animal and crop husbandry are needed to close the significant productivity gaps, particularly in sub-Saharan Africa, mitigate and build resilience to climate change. On the other hand, in Europe and other temperate production systems, mitigating greenhouse gas emissions and increasing production systems' resilience to drought and other environmental stresses should be the highest priority. The United Nations acknowledge and seeks to address these challenges by 2030 as reflected in the SDGs (Goals 1-3, 6, 12-15). However, any attempt to reduce GHG emissions from agriculture is unlikely to be adopted unless production efficiency is maintained if not enhanced. Henceforth, the tetrahedral challenge of food security, climate change, rural development and poverty alleviation debacle must be resolved using knowledge-based sustainable agricultural approaches to enhance crop and animal productivity, ensure better soil health, and improve ecosystems service provision. The above challenges call for a new paradigm to find new ways to produce agricultural goods sustainably.

To this end, various propositions have come up, including the concept of sustainable intensification, which aims at producing more food using fewer resources with low environmental impact (THE ROYAL SOCIETY, 2009). Accordingly, sustainable production of agricultural goods is a significant pillar of the knowledge-based bio-economy agenda pursued by the European Union, USA, Japan, India and Brazil (CELIKKANAT OZAN & BARAN, 2014). The concept of ecological intensification, which involves using modern technologies to produce socio-economic goods and multiple environmental services simultaneously, has been forcefully proposed (FRANZLUEBBERS *et al*., 2011; LEMAIRE *et al*., 2015; SCHIERE *et al*., 2002; SULC & TRACY, 2007). This concept is pivoted on the diversification at the field, farm, landscape and/or regional scales to optimize agricultural productivity per unit natural resource (LEMAIRE *et al*., 2014). Such diversification might include re-introducing crop-livestock systems, increasing crop diversity at all scales and rotation, and introducing forage crops and grasslands into crop rotation cycles. These systems' workability might vary depending on environmental and managerial conditions, making research on their economic and ecological viability necessary.

1.3 Relevance of grassland systems

Grasslands and savannahs comprise 26–40% of the earth's land area and constitute 80% of the worlds' agricultural lands (DUDLEY *et al.*, 2020). Grasslands form a significant source of goods and services such as food, forage and energy, feeding over 800 million of the world's population (WHITE *et al.*, 2000). However, almost all (92%) natural grassland on the earth have been converted to human use, mostly grazing and cropland production (STERLING & DUCHARNE, 2008). The high inherent SOM content of grasslands supplies plant nutrients, increases soil aggregation, limits soil erosion and increases cation exchange and water holding capacities (CONANT & PAUSTIAN, 2002). Accordingly, grasslands have a greater propensity to store SOC than croplands (CONANT, 2010; FRANZLUEBBERS, 2010; GUO & GIFFORD, 2002). However, grasslands are sensitive to perturbations and respond rapidly to manipulations (BLAIR *et al.*, 2014). For example, conversion of grasslands to arable has been shown in a meta-analysis to reduce carbon stocks by 59% (GUO & GIFFORD, 2002). Ploughing can also result in large fluxes of nitrous oxide and carbon dioxide (REINSCH *et al.*, 2018). Thus, changes in management or land use type in tropical and temperate grasslands could have an enormous impact on global N and C cycles.

The soil is a significant reservoir for C and N, storing about three times more C and N than the atmosphere (LAL, 2004). Carbon storage in grassland soils depends on the carbon cycle constituents, as shown in Figure 1.1. The four major factors, including biomass input and offtake and the retention and stabilization of the SOM (WHITEHEAD, 2020), influence C balance considerably. Whereas the amount of input from roots and exudates is a function of species type and soil nutrient availability, SOC retention and stabilization depend on soil management, soil type, and organic input quality. Carbon sequestration, which refers to the natural and deliberate processes by which CO_2 is either removed from the atmosphere or diverted from emission sources and stored in the ocean, terrestrial environments (vegetation, soils, and sediments), and geologic formations (SUNDQUIST *et al.*, 2008), provides an opportunity for agriculture to offset non-preventable emission sources and achieve climate neutrality. However, many practices, including reducing the length of fallow periods, over-grazing, excessive aboveground biomass export and grassland termination or renovation, have reduced soil organic matter, water holding capacity and nutrients in many ecosystems (JONES & DONNELLY, 2004). Consequently, grasslands and savannahs have begun to contribute to greenhouse gas emission budgets (CONANT, 2010; RIDDER, 2007).

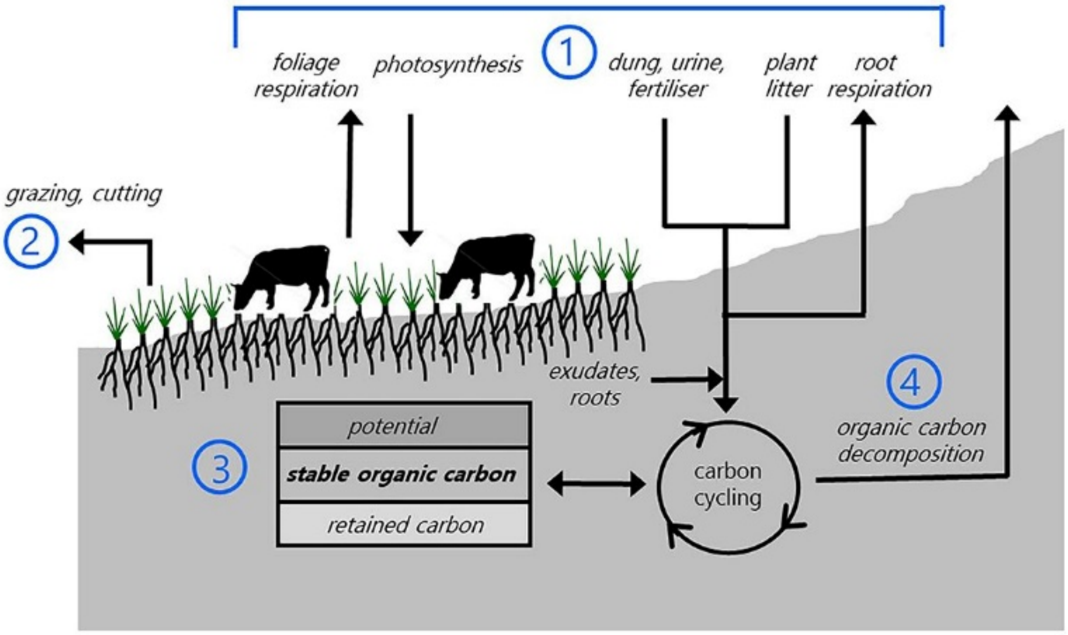

Figure 1.1. Grassland C cycle, showing the major constituents 1, 2, 3 and 4. Adopted from (WHITEHEAD, 2020)

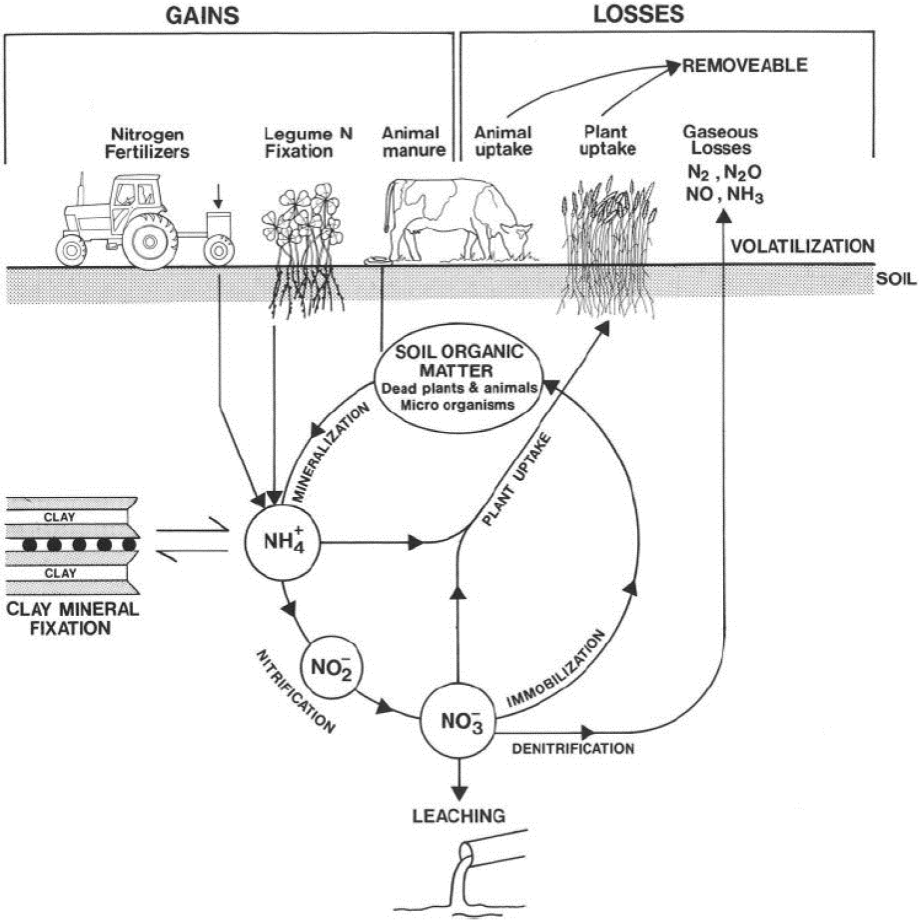

Figure 1.2: Typical N cycle in a temperate grassland ecosystem (Source; (BUCKTHOUGHT, 2013)

A typical grassland N cycle is strongly regulated by complex interactions between soil, plants, and livestock (Figure 1.2). These interactions are mediated by prevailing climatic conditions and the farmer's management decisions, resulting in N gains or losses. These heterogeneous factors include N source and amount, temperature and rainfall, soil moisture and microbiome, as well as botanical factors (FIRESTONE & DAVIDSON, 1989; LEDGARD, 2001; VOGLMEIER *et al.*, 2019). Accordingly, N losses from grasslands (intensively managed and grazed grasslands) are associated with the highest uncertainty (FLECHARD *et al.*, 2007; VAN DER WEERDEN *et al.*, 2020; WHITEHEAD, 2020). Previous studies, as summarized by (SELBIE *et al.*, 2015), showed that N losses from urine N applied to European grasslands ranged 1–27% via volatilization (NH_3), 0–14% through nitrification and denitrification (N_2O and N_2) and 13–54% via NO_3 leaching while N immobilization and uptake by plants ranged 13–54% and 19–65%, respectively.

1.4 Challenge: increasing inputs without destabilizing agricultural production systems

Nitrogen inputs are crucial to the profitability of most agricultural systems. However, the addition of N beyond the absorptive capacity of an ecosystem, as is the case in many temperate agricultural regions, results in excess N, which get lost to the environment via leaching, volatilization, nitrification and denitrification. Currently, N application to global ecosystems is very high (> 100 kg N ha^{-1}) and is projected to increase by a factor of 2.5 in the next century (GALLOWAY *et al.*, 2004; LAMARQUE *et al.*, 2005), as agriculture is intensified to meet the increasing global food demand. Furthermore, nitrogen losses via leaching are a threat to groundwater bodies, thus potentially polluting drinking water. For these reasons, the deposition of reactive N is a significant threat to ecosystems' functioning and sustenance (FIELD *et al.*, 2014). The increased deposition of reactive N will further increase N_2O emissions from agricultural systems. Indeed, increased adoption of farm practices that decouple C and N cycles decrease C and N ratio, reduce C residence time in soils have heightened the negative impact of agriculture on the environment (LEMAIRE *et al.*, 2015). These worrying trends have rendered most agroecosystems less resilient to climate change and have negatively impacted soil health, air quality, and underground water.

Introduction of grassland leys into arable cropping systems (e.g. legume-based) could improve soil structure and soil fertility, control weeds, improve C stocks, enhance productivity, reduce water runoff and soil erosion and increase biodiversity. Although grazing converts consumed plants into CO_2 and other greenhouse gases, multiple studies have shown that grazing can often lower net ecosystem carbon emissions and promote soil carbon storage (MCSHERRY & RITCHIE, 2013), especially in dry ecosystems (CHEN *et al.*, 2015). However, the effect of grazing on SOC is inconclusive due to inadequate data. The introduction of grasslands in farm systems may reduce N losses if N input and stocking rate, proper timing of tillage or good rotation management (KHALIL *et al.*, 2019). As the most geographically extensive grassland use today, well-managed grazing systems can significantly influence global soil carbon storage (BAGCHI & RITCHIE, 2010). Nevertheless, the impact of grasslands on N loss mitigation may depend on grassland types, environments and managements (LEMAIRE *et al.*, 2015).

1.5 Justification of the studies

Plants play a significant role in C and N cycles (Figure 1.1 & 1.2), regulating C and N supply to soils via litterfall, root decay and root exudates, controlling microbial population dynamics in most soils, yet their impact may vary depending on the vegetation type (YANG *et al.*, 2019). Most ruminant livestock systems worldwide rely on a wide range of grasses, legumes and herbs. Adopting diverse forage species in intensified grassland systems could enhance the sustainable production of goods and ecological services. However, it is necessary to identify trade-offs by considering environmental carrying capacity (LEMAIRE *et al.*, 2015), which might differ from one location to another. FREDEEN *et al.* (2013) contend that emission of GHGs is system-related, and the type of feed used in pasture and confinement systems has potential implications on both nutrient excretion and total GHGs emissions (ARSENAULT *et al.*, 2009). Therefore, such forage species should have the capacity to improve animal performance while enhancing their ecological efficiency. However, forage breeding lines are often developed and promoted without considering their ability to improve ecological efficiency.

Grasslands have a high capacity to sequester soil C and contribute to the global effort to mitigate greenhouse gas emissions (MINASNY *et al.*, 2017; SOUSSANA *et al.*, 2010). The adoption of improved grasslands species was reported to have the highest C sequestration rate than other recommended practices (LAL, 2008). Despite the lack of data on carbon budget, various model simulations (CIAIS *et al.*, 2009) have observed that Africa could be acting as a net carbon sink. BOMBELLI & HENRY (2009) suggested a more profound knowledge of mitigating climate change on the continent through carbon sequestration. Enhancing the capacity of forage production systems to sequester carbon could help reduce livestock production's carbon footprint. However, poor soil fertility management, primarily seen in land overuse without nutrient replenishment, could derail the C storage potential of soils in SSA. Under such harsh soil and management conditions, plants with high nutrient use efficiency could be more ecologically efficient. Plants that produce greater root mass and produce a litter with slower decomposition rates tend to store more SOC than those that produce lower root mass and produced rapidly decomposing litter. Recent studies have also shown plants' roles in stabilizing soil organic carbon (ADAMCZYK *et al.*, 2016, 2017; CHOMEL *et al.*, 2016; SHANMUGAM *et al.*, 2018). Soil C cycling, therefore, its mean residence time, is regulated by plant residue biochemistry (MARTENS, 2000) through chemical complexing processes between polyphenols, lignin and soil particles (JONES & DONNELLY, 2004).

The loss of N to the environment is an ecological cost and an economic cost to the farmer. The measures proposed to reduce N losses have targeted the soil, using process inhibitors such as nitrification or urease inhibitors. Other measures include manipulating functional pasture structure, managing feeding to reduce N excretion, timing grazing and adopting fertilizer strategies to prevent N deposition at high emission moments (DE KLEIN et al., 2019; SELBIE et al., 2015; WAGNER-RIDDLE et al., 2020). The use of chemical nitrification inhibitors being very effective, reducing N losses up to 56% (SELBIE *et al.*, 2015); however, their applicability has been fraught with practical difficulties at the paddock/farm scale (SELBIE *et al.*, 2015). Moreover, traces of the chemicals have been reported as residues in milk (DANAHER & JORDAN, 2013), making their use in organic livestock systems difficult.

Over the past few years, Europe has developed some strategies and policies to reduce N accumulation in soils. However, it appears these efforts have not yielded significant results as, for example, a substantial amount of groundwater bodies in northern Germany remain in a 'bad state' (BIERNAT *et al.*, 2020). Moreover, the agricultural sector appears to respond slowly to the adoption of these technologies. The German government, for instance, has set an aggressive agenda to reduce N surplus to a maximum of 70 kg ha^{-1} between 2028 and 2032, with a long-term target of 50 kg ha^{-1} (STALLMANN, 2014). However, the complexity of the mitigation strategies and cost could be deterrents to adopting climate-smart agricultural practices. Therefore, the urgent need for innovative (LONG *et al.*, 2016) low-cost and easy-to-use climate-smart strategies to mitigate N_2O emissions from agricultural systems is imperative.

Mixed pastures have a higher propensity to reduce N_2O emission due to faster growth and rapid resource acquisition (ABALOS *et al.*, 2014; KRAMER-WALTER *et al.*, 2016; NYAMEASEM *et al.*, 2021). Moreover, legume use in pastoral systems to reduce mineral N use has been highly recommended, as the biologically fixed N bypasses the soil mineral N pool resulting in low N losses (FUCHS *et al.*, 2020). Besides atmospheric nitrogen fixation, forage mixtures containing legumes out-yield monocultures and have lower carbon footprints (KHATIWADA *et al.*, 2020). This over-yielding effect is because mixed pastures have a higher capacity to produce more biomass due to efficient use of resources even under unfavourable climatic conditions (NOBILLY *et al.*, 2013; WOODWARD *et al.*, 2013). Furthermore, plants rich in secondary metabolites may inhibit the nitrification process via root exudates, resulting in low N losses (DE KLEIN *et al.*, 2019). Besides, acquisitive species are aggressive and can remove excess N from soils, reducing N substrate available for microbial transformations (ABALOS *et al.*, 2014). When well harnessed, mixed or diverse swards containing some useful herbs could enhance nutrition and welfare and provide additional ecological services such as supporting other life forms to ensure biodiversity in the ecosystem (VILLALBA *et al.*, 2019). Based on the foregone considerations, it suffices to speculate that the impact of a forage species on the environment will depend on their nutrient use efficiency, phenology and effect on the rhizosphere. However, the effects of forage species on the environment are inconsistent, and the driving factors are not well understood (DE KLEIN *et al.*, 2019) due to insufficient data from diverse environmental and management conditions.

1.6 Research objectives and hypotheses

To assess the potential ecological efficiency of diverse pastures and management systems, we conducted two field studies and a pot experiment under tropical and temperate settings. The objectives of the studies were:

(i) To assess the impact of different land-use types and plant functional groups on soil carbon storage and explore plant and soil factors contributing to soil carbon storage differences in tropical forage production systems. The study tested the following hypothesis:

Hypothesis: Plant functional diversity affects soil C stocks under similar environmental and management conditions.

(ii) To investigate the impact of pasture diversity and irrigation on N_2O emission and emission factors and identify factors that drive N_2O emission in legume-based pastures. The hypotheses tested include:

Hypothesis: Pastures with higher functional diversity emit less N_2O-N compared with less diverse swards.

(iii) To assess the N_2O emission mitigation potential of Lancelot plantain in northern Germany and explore the effect of belowground resource allocation N_2O emission and microbial response under varying soil N levels. The tested hypotheses were:

Hypothesis: Pastures rich in Lancelot plantain emit less N_2O-N compared with perennial ryegrass-rich swards.

1.7 The experimental study sites

The nature of environmental impact by agriculture varies across the globe. Therefore, there is no one-method-fit-all solution to mitigate the effects of agriculture on the environment around the world. Contrasting climatic factors, soil properties, and vegetation have long been the basis for predicting changes in C and N cycles. However, where climatic and soil properties are relatively uniform at the farm level, land use differences may influence C and N's quantity and quality added to the soil. These variables cause plant-soil feedbacks to regulate soil carbon and N cycling, which can cause local variations in C and N fluxes (CHEN *et al.*, 2018; POST *et al.,* 1982; SAIZ *et al*., 2012). Hence, to develop site-specific solutions (GAO & CARMEL, 2020) and concepts for more extensive research in the future and capture site-specific realities, case study experiments are imperative.

1.7.1 CSIR research farm, Pokuase, Ghana

Field experiments that measured soil carbon stocks from pastoral systems are uncommon in SSA. Thus, the C sequestration potential of grasslands in SSA is estimated using a broad scale model-based approach, extracted from global studies and limited data points. As a result, factors responsible for variations in carbon storage at the farm scale level are not well defined. Given that such long-term experiments are scarce, particularly in SSA, and results emanating from such a facility could be widely applied within similar agroecosystems, this site was chosen for the case study.

The CSIR research fields in Pokuase, located in southern Ghana, originated from the else-while state farms owned by the Ghana government. When the state farm's operations ceased, the arable lands were then allocated to various state research institutions in agricultural research and extension services, including the Crop and Animal Research Institutes of CSIR. Some fields have been used for arable crop experimentations and production annually, while some portions often undergo fallow when not in use. Regular tillage, land fertilization (organic and inorganic sources), and general cultural practices are all management strategies. In addition, lands were set aside for pasture seed multiplication and agronomic experimentation where regular tillage, pasture crop rotation and soil fertility were practised. Also, a portion of the land was set aside for pasture germplasm maintenance, referred to as the ‘Pasture museum’, where specific plot sizes of 4 x 4 metres were maintained to house live forage species brought from all over the world. Most of the designated plots have harboured the same species with very few exceptions since their establishment in 1966. General weed control on the plots to maintain the plots' outlines and integrity and regular harvesting were the primary management practice.

1.7.2 University of Kiel Organic Research Farm, Lindhof, Germany

The Lindhof farm, belonging to the Institute of Crop Science and Plant Breeding at Kiel University and managed by the grass and forage science /Organic Agriculture (GFO) department, is a certified organic experimental farm producing beef, pork and arable crops. Milking about 100 organically pasture-based spring calving Jerseys and Jersey/EBI crossbreeds annually, the farm is also known for low-input milk production. Furthermore, being one of the EuroDairy pilot farms, the farm engages in several regional EIP-projects (e.g. "Smart-Grazing", "Catch-crops for forage production", and "Weidemanager Schleswig-Holstein"). Besides, this farm is dedicated to low-input and organic farming research, emphasizing the N and energy use efficiency of various crops and farming systems.

In recent years, Lindhof research farm is integrating secondary legumes like red- clover and birdsfoot trefoil as well as herbal forage plants, e.g. Lancelot plantain, chicory, sheep's burnet and caraway (all deep routing), into their forage systems to increase water use and plant nutrient-use efficiency as well as contribute positively to soil fertility. Furthermore, integrating these species into classical ley consisting of perennial ryegrass and white clover improves forage uptake during the grazing season and forage quality and biodiversity. The availability of these diverse pasture resources at this farm and the fact that the research results could have broader regional applicability necessitated its choice for this doctoral study.

1.8 Thesis structure

Three field-based experiments were conducted between 2018 and 2020 to address the objectives set out for this thesis. In the first part, this thesis assessed the outcome of C inputs and outputs across different arable and permanent systems consisting of diverse plant functional groups of a tropical savannah in southern Ghana after more than 50 years of consistent land use (*Chapter* 2). Next, the hypothesis that relatively low N_2O emission factors characterize legume-based N sources was tested across a gradient of plant species diversity and under irrigation in Lindhof experimental farm (*Chapter* 3). Finally, the effects of N availability and sward differences on N_2O emission were tested in a greenhouse experiment, and the impact of biomass C/N partitioning belowground and rhizospheric microbial enzyme activity on N_2O emission and emission factors was explored (*Chapter* 4). The overall findings and their implications were concluded at the end of this thesis in *Chapter* 5. The thesis has been organized as a cumulative thesis. Accordingly, *Chapters* 2, 3 and 4 have been presented as independent articles; therefore, the introduction, site details and synthesis might be repeated. The format and styles of *Chapters* 2, 3 and 4 followed the guidelines of journals to which the manuscripts were submitted. The articles are listed below:

Chapter 2: NYAMEASEM, J. K., REINSCH, T., TAUBE, F., DOMOZORO, C. Y. F., MARFO-AHENKORA, E., EMADODIN, I. AND MALISCH, C. S. (2020). Nitrogen availability determines the long-term impact of land-use change on soil carbon stocks in grasslands of southern Ghana, published in *SOIL*, 6, 523–539, 2020. https://doi.org/10.5194/soil-6-523-2020

- This chapter reports the outcome of C inputs and outputs across various plant functional groups in arable and permanent systems of a tropical savannah after more than 50 years of consistent land use. The hypotheses tested included the following: (i) Soil C stocks in sub-Saharan Africa are profoundly affected by the type of land use management, (ii) Soil macronutrients and CTs can predict long-term changes in SOC stocks of tropical grasslands, and (iii) Plant functional groups influence the relationship between SOC and soil N. Accordingly, the study measured soil organic carbon (SOC) stocks (0–30 cm depth) from arable crop fields, grazed–seeded grassland, cut–use permanent crops and native grassland. Additionally, the study explored how soil nutrients and secondary plant metabolites (condensed tannins) impact SOC's impact using the native grassland as the baseline. The results provide the theoretical that improved soil fertility management and the use of tannin-rich plants can promote long-term SOC storage in the savannah ecological region.

Chapter 3: NYAMEASEM, J. K., MALISCH, C. S., LOGES, R., TAUBE, F., KLUß, C., VOGELER, I. AND REINSCH, T. (2021). Nitrous Oxide Emission from Grazing Is Low across a Gradient of Plant Functional Diversity and Soil Conditions, published in *Atmosphere*, *12*, 223, 2021. https://doi.org/10.3390/atmos12020223

- This chapter explored the potential of legume-based mixed pastures to mitigate nitrous oxide emission under grazing pressure. Specifically, the study tested the hypotheses that (i) grassland diversity affects N_2O emission under grazing stress, (ii) botanical and environmental factors determine N_2O emission from soils, and (iii) N_2O emission factor (EF) for cow excreta deposited on irrigated pastures is lower for systems with the legume as a sole N source compared with the IPCC default. Accordingly, this study monitored N_2O emissions for a minimum of 100 days and up to two growing seasons across a gradient of plant species diversity. Emissions were measured from grazed pastures and applied urine and dung patches using the static chamber method under ambient and irrigation conditions. The study subsequently reported annual N_2O emissions from grazed legume-based pastures and disaggregated N_2O emission factors for northern Germany, as affected by grassland type, the season of excreta deposition and irrigation. Additionally, the study reports environmental and botanical factors that drove N_2O emissions from the grasslands.

Chapter 4. JOHN K. NYAMEASEM, ENIS B. HALIMA, CARSTEN S. MALISCH, BAHAR S. RAZAVI, FRIEDHELM TAUBE, THORSTEN REINSCH. Nitrous oxide emission from forage plantain and perennial ryegrass swards is affected by resource allocation dynamics belowground.

- The study reported a greenhouse experiment that explored the potential of Lancelot plantain inclusion in pastures as a tool to mitigate N_2O emission under northern Germany environmental conditions. The study tested the hypotheses that (i) N_2O emission from Lancelot plantain-rich swards is lower than from perennial ryegrass monoculture swards due to their expected nitrification inhibiting effect, and (ii) that microbial enzyme expression is highly associated with Lancelot plantain proportion in the swards as well as with C/N ratio belowground. Using soils from Lindhof experimental farm, three grasslands (*Plantago lancelota,* PL; *Lolium perenne,* LP; and LP-PL mixed swards) established in pots, which received 0, 150 or 300 kg N ha^{-1} equivalents of fertilizer, were monitored for N_2O-N emission for three months. Furthermore, additional parameters, including dry matter yield, carbon and nitrogen assimilation above- and belowground, and microbial enzyme (leucine aminopeptidase) activity as a proxy for soil microbial activity, were measured. Invariably, the results suggest that resource allocation dynamics belowground have a higher dominance on N_2O emission mitigation from soils rather than forage plantain itself.

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Nitrogen availability determines the long-term impact of land use change on soil carbon stocks in grasslands of southern Ghana

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CHAPTER 2

Nitrogen availability determines the long-term impact of land-use change on soil carbon stocks in grasslands of southern Ghana

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This chapter reports C inputs and outputs across various plant functional groups in arable and permanent systems of a tropical savannah after more than 50 years of consistent land use. This chapter is an original work of this thesis, which involved the first author from the experimental planning, the field and analytical work to the article's writing.

Abstract

Enhancing the capacity of agricultural soils to resist soil degradation and mitigate climate change requires long-term assessments of land-use systems. However, such long-term evaluations, particularly regarding low-input livestock systems, are limited. In the absence of suitable long-term experiments, this study assessed the outcome of C inputs and outputs across an array of plant functional groups in arable and permanent systems of a tropical savannah after more than 50 years of consistent land use. Soil samples were taken (0–30 cm depth) from arable crop fields, grazed–seeded grassland, cut–use permanent crops and native grassland. Soil organic carbon (SOC) stocks ranged from 17 to 64 Mg SOC ha^{-1} (mean ± sd = 32.9 ± 10.2 Mg ha^{-1}). SOC stocks were lower for grazed–seeded grassland relative to cut–use grass, legume trees and shrubs. Accordingly, while the conversion of the native grassland to grazed pastures caused an estimated loss of 44 % of SOC over the period, the conversion to woody legumes resulted in slight (5 %) incremental gains. Within sown systems, nitrogen (N) availability seemed to be the most critical factor in determining the fate of the SOC stocks, with the soil N concentration and SOC being highly correlated ($r = 0.86$; $p < 0.001$). In total, N, P and K were significant predictors of SOC density in the soils. Moreover, secondary plant metabolites in legumes, namely tannins, were identified as impacting SOC. The results from this study provide the

theoretical basis for testing the hypothesis that improved soil fertility management and the use of tannin-rich plants can promote long-term SOC storage in the savannah ecological region. Our study also shows the potential of legume tree/shrub forage species as an environmentally sustainable land use option to mitigate agricultural CO_2 emissions from low-input livestock systems in the grasslands of southern Ghana.

Keywords: grassland, land-use change, legumes, proanthocyanidins, soil carbon sequestration, soil properties, sub-Saharan Africa

2.1 Introduction

Increments in increased carbon (C) sequestration rates in soils of 0.4 % per year have been suggested as a means of compensating for the global emissions of greenhouse gases from anthropogenic sources (CHABBI *et al*., 2017). On a global scale, grasslands sequester around 0.14 Mg C ha^{-1} $year^{-1}$, thus storing 685 Gt C in the upper soil (1 m depth). This C pool size is nearly 50 % more than that of forests (346 Gt C) and 70 % more than wetlands (202 Gt C; (CONANT *et al*., 2017; GRACE *et al*., 2006; GOBIN *et al*., 2011). About 60 % of all grasslands occur in the tropics, containing 10 %–30 % of the global soil C stocks (CAQUET *et al*., 2012). Besides their potential for mitigating climate change, increased soil C sequestration could enhance ecological efficiency and deliver other related ecosystem services, especially food, water and biodiversity (CONANT *et al*., 2017; STRINGER *et al*., 2012). However, large areas of native grasslands worldwide have undergone a substantial use intensification or have been converted into pasture and croplands (STERLING & DUCHARNE, 2008; TAUBE *et al*., 2014). This action has led to either reduced C sequestration rates or net losses of soil organic C (SOC; CREWS & RUMSEY, 2017; JOHNSTON *et al*., 2009; REINSCH *et al*., 2018). This change is also visible in sub-Saharan Africa, where overgrazing and other land uses affect the C cycle (GRIECO *et al*., 2012) and might constrain the attainment of the 4 ‰ agenda (MINASNY *et al*., 2017) in the sub-region.

Conversion of natural to managed ecosystems generally results in depleted soil C stocks. For example, the conversion of native grasslands to crop production has resulted in an approximately 50 % loss of SOC in global grassland ecosystems (LAL, 2018) as a consequence of destabilizing stored SOC. Adequate organic matter (OM) input into soils is necessary for increased C sequestration. However, sequestered C is sensitive to management and land-use changes, particularly grazing, changes in species composition, and mineral nutrient availability

(CONANT *et al.*, 2017). Nevertheless, management practices that increase the supply of quality OM could promote C storage in soils. Accordingly, soil nutrients have implications for plant primary productivity and ecosystem functioning (MARQUES *et al.*, 2016; Post et al., 2012); however, their exact effects on C sequestration, particularly in grasslands dominated by C_4 species, are not well understood (MILNE *et al.*, 2016). For example, the impact of N fertilization on SOC dynamics remains controversial because of its dependence on other parameters such as soil pH, available phosphorus (P) and potassium (K) and the frequency of tillage management operations (KHAN *et al.*, 2007; LAL, 2008; REINSCH *et al.*, 2018).

The C input into soils derived from plant residues and organic manures and the carbon's stabilization in the soil are essential for increased soil C sequestration. Some SOC stabilization mechanisms that have been proposed include physico-chemical protection of SOC by micro- and macroaggregates, spatial separation of SOC from decomposers by encapsulation, occlusion and hydrophobicity and mineral–organic associations (QUESADA *et al.*, 2020; KLEBER *et al.*, 2015; SONG *et al.*, 2018). Moreover, plant secondary metabolites in both above- and below-ground biomass have recently been discussed to have an impact on both C and N cycles, with the potential to either increase or reduce C immobilization rates in soils (ADAMCZYK *et al.*, 2016, 2017; CHOMEL *et al.*, 2016; HALVORSON *et al.*, 2011; KAGIYA *et al.*, 2019; KRAUS *et al.*, 2003; TAMURA & THARAYIL, 2014). Condensed tannins (CTs), for example, enter the soil as leachates and via decomposed litter from plant leaves and roots (HÄTTENSCHWILER & VITOUSEK, 2000). Although most forage species naturally contain relatively low levels of CTs, remaining suitable for livestock feeding (MUELLER-HARVEY *et al.*, 2019), the concentration of CTs may reach potent levels in the long term because CTs are recalcitrant and could remain in the soil for decades (TAMURA & THARAYIL, 2014). However, CTs' role in mitigating CO_2 emissions, particularly from tropical soils, has received minimal attention.

In livestock systems, where a large part of the net primary production is exported from the soil as hay, silage or livestock products, the below-ground biomass is a significant C input source. Mechanisms driving SOC sequestration in livestock systems, particularly low-input systems in the tropics, are not well understood due to lack of research data and sometimes conflicting results, even for such important factors as grazing (MCSHERRY & RITCHIE, 2013). Previous studies have shown mixed effects of grazing on soil C, including positive (REEDER & SCHUMAN, 2002), neutral (SHRESTHA & STAHL, 2008), or adverse effects (PEI *et al.*, 2008).

Due to the complexity of the different above-described controlling factors on SOC stocks, long-term experiments are ultimately necessary to validate the assumptions that have been made. In the absence of long-term experiments in sub-Saharan Africa designed to answer this research question, we selected a research farm that has cultivated and managed plots of different plant species according to the same principles for 50 years. The research farm, located in the southern savannah belt of Ghana, was previously reserved government land. Around 50 years ago, parts of it were simultaneously converted to farmland and research plots. The site offers the unique opportunity to test the long-term effects of converting native grassland to agricultural land using the current SOC and nutrient stocks of the undisturbed grassland, which have not been converted within these 50 years, as a reference baseline. The homogeneous environmental conditions across the entire sampling area would enable estimation of the divergence in SOC and nutrient stocks, due to the different land use changes, when compared to the hypothetical SOC and nutrient stocks represented by the undisturbed grassland. Thus, the main aim of this study was to assess the impact of long-term land use practices on soil C storage and the potential role of tannin-rich forages and soil nutrient status. In detail, the current case study was conducted to test the following hypotheses:

1. Soil C stocks in sub-Saharan Africa are profoundly affected by the type of land use management.
2. Soil macronutrients and CTs can predict long-term changes in SOC stocks of tropical grasslands.
3. Plant functional groups influence the relationship between SOC and soil N.

2.2 Materials and methods

2.2.1 Site characteristics

The study was conducted at a research farm of the Council for Scientific and Industrial Research (CSIR), Ghana, located at 5°70′ N, 0°29′ W and 49 m above sea level (a.s.l), some kilometres from Accra, Ghana (Figure 2.1). The mean monthly temperature ranges from 21 to 31 °C, and the monthly rainfall ranges from 13 to 205 mm (annual rainfall is approximately 800 mm). The principal rainy season is from April to mid-July, with a minor rainy season in October (Figure 2.2). Thus, the climate is moist semi-arid, with a growing period lasting 120–180 d (GHANA METEOROLOGICAL AGENCY, 2019). The surface lithology is non-carbonate sedimentary, with coarse sandy loam soils belonging to the Haplic Acrisol group (IUSS WORKING GROUP WRB, 2014). The soil at the site had the following features at the 0–

10 cm soil depth: 80.34 % sand, 12.64 % silt, 7.02 % clay, 5.86 pH (water), 0.133 % N, 2.10 parts per million (ppm) available P, 55 ppm available K and 2.61 % OM (BARNES, 1999). The native land cover of the study location is a tropical grassland savannah.

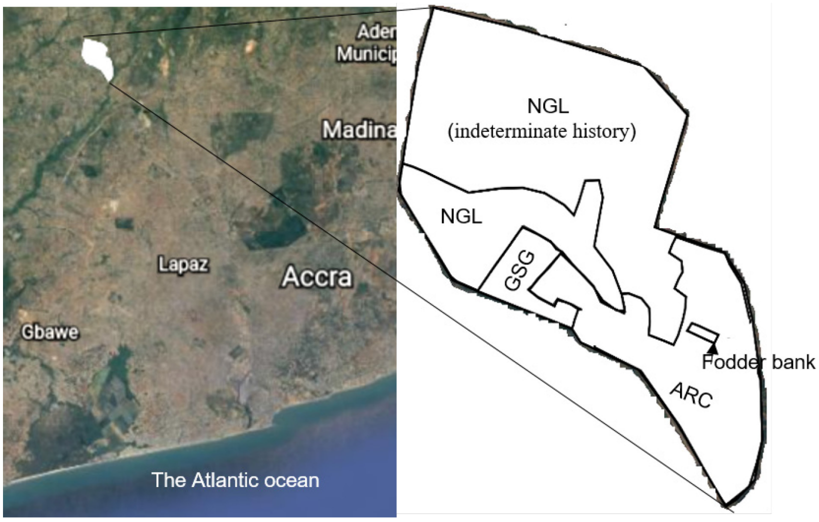


Figure 2.1: Location and plan of the study site. The native grassland (NGL) remains uncultivated since the farm was established in 1966. The portion of NGL labelled "indeterminate history" was excluded from the study due to the high uncertainty associated with its history. The fodder bank consisted of cut-use grasses (CG), legume herbs (CLH), legume tree/shrubs (CLTS) and non-legume tree/shrubs (CNLTS); Map from ©Google Earth (2020).

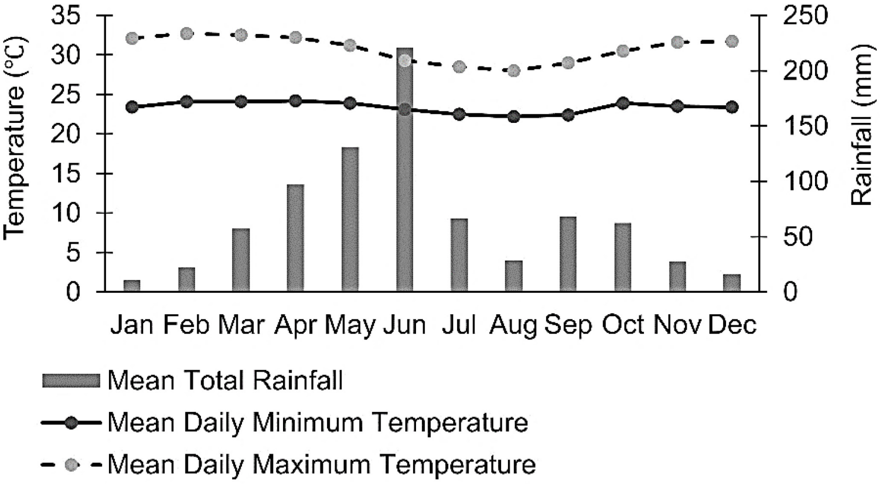


Figure 2.2: Climate (30-year average) of the study area (Data source: GHANA METEOROLOGICAL SERVICES DEPARTMENT, 2019)

The research farm, located on the grazed–seeded grasslands (GSGs), keeps Sanga cattle, a cross between the humped Zebu-type cattle and the local West African Shorthorn known for their resistance to trypanosomiasis (adult weight range of 300–330 kg), and Djallonké sheep (adult weight range of 25–37 kg) that are managed semi-intensively. These animals are grazed rotationally on seeded pastures during the rainy season (April–October) and fed on conserved fodder harvested from arable fields and a fodder bank. While the exact stocking density is not recorded, typical stocking capacity around that region has been reported to range from 3.5–5 LU ha^{-1} (TIMPONG-JONES *et al.*, 2013). Compared to that, the native grasslands (NGLs) are infrequently grazed in a nomadic fashion, and no records about their stocking densities are recorded, yet grazing is limited to the dry season when feed is scarce at the GSG plots.

Table 2.1. Brief description of land-use types at the study site

Land-use type	Description
Native grassland (field) (NGL)	Dominant plant species included *Panicum maximum, Cyperus spp, Talinum triangulare* and *Panicum decumbens* and intermittent occurrence of shrubs, including *Acacia sp.* and *Azadirachta indica*, lightly grazed by sheep and cattle; aboveground biomass yield is 5.7 – 7.2 t ha^{-1} (TIMPONG-JONES *et al.*, 2013); uncultivated since the last 50 years
Arable crop production (ARC) (field)	*Three fields; sown crops: Manihot esculenta*, *Arachis hypogea*, *Zea mays* and *Vigna unguiculata*, as well as fodder grasses and legume herbs; two fields utilized zero tillage, while one was fully tilled; only one of the fields received fertilizer
Grazed-seeded grasslands (GSG) (field)	Single-species pastures -*Brachiaria brizantha*, *B. ruziziensis* and *Digitaria decumbens*; grazed rotationally by Djallonke sheep (25-37 kg adult weight) and Sanga cattle (300-330 kg adult weight).
Cut–use forage plots	Monoculture (25-30 m^2); 38 species of grasses (CG), 11 species of legume herbs (CLH); seven species of legume trees/shrubs (CLTS) and three species of non-legume trees/shrubs (CNLTS), respectively, no soil amendments. The plots were weeded to maintain plot purity and harvested twice in the wet season and once in the dry season. Prostate and erect grasses and herbaceous legumes were harvested at 6-10 cm and 10-20 cm high, respectively, while shrubs were defoliated at 30 cm high. Annual dry matter yields (mean±sd) from the forages were 8.63±3.57, 4.08±1.41 and 6.53±2.17 Mg ha^{-1} for shrub/trees, legume herbs and grasses, respectively.

The site was selected due to its suitability for agriculture, its proximity to the capital city and its vegetation and climate, representing the most significant grassland type of Ghana (Guinea savannah). Ostensibly, the site had a uniform land usage until parts were converted for different uses in 1966 and subsequent years (Figure 2.1). Briefly, the land uses we encountered comprised arable field crops and grazed–seeded grasslands (both at field scales with at least 1 ha) and a fodder bank that also served as a forage botanical garden housing plant species brought from all over the world. Our study considered 59 species relevant to forage production (Appendix A Table A1) and represented four plant functional groups: cut–use grasses (38 species), cut–use legume herbs (11 species), cut–use legume trees and shrubs (seven species) and cut–use non-legume trees and shrubs (three species) at plot scales (25–30 m^2). Several species' accessions were present, and we sampled 72 plots from the 59 species. The management practices associated with the land use types are summarized in Table 2.1.

2.2.2 Soil sampling

Field/plot selection was based on the following two main criteria: (I) that the field was established in 1966 and (II) that the management remained relatively stable over the 50-year time frame. Information regarding the management of fields and biomass productivity of the species was obtained from the farm documentation. Plots/fields that underwent some management changes or were re-established for any reason were omitted from the study. Consequently, there were no replicated plots, as in a traditional agricultural experiment, in the case of the cut–use forage species to control for variation. Hence, we categorized the forage species into functional groups, with each functional group's species constituting a pseudo replicate.

Soil samples were taken from the native grasslands and arable crop fields by zone-based composite sampling, where we divided the fields into three zones (northern, central and southern). We randomly selected three paddocks out of the nine for soil sampling from the seeded-grazed paddocks. We collected 10–20 subsamples (depending on the zone's size), spread evenly across each zone in a zigzag pattern, to constitute a composite sample, with each composite soil constituting a replicate. The sampling procedure for the plot-scale samples followed the recommendations by (SAIZ & ALBRECHT, 2016). Sampling locations were determined by roughly locating the first at the centre of the field, with three replicates laid out according to a pattern of three axes separated by 120∘ to a primary axis pointing north. Replicates were selected along these axes at an approximate midpoint between the centre of the field and its boundaries.

Before any sampling, surface litter was removed by hand. Then, soil samples were taken for each sampling plot or field (0–30 cm soil depth) using a soil probe of 1 in. (25.4 mm) diameter. Replicate samples obtained were bulked and thoroughly mixed, and a representative sample was taken in a zipped polythene bag for analysis. Due to the higher number of accessions, the total number of soil samples obtained from the cut–use forages was 72, derived from 59 species. For the estimation of soil bulk density, three to eight sets of soil samples were taken from each experimental unit (replicate plots/fields), depending on size, using a stainless steel core sampler at depths of 0–5, 5–10, 10–15, 15–20 and 20–25 cm. Soil bulk density and C stocks were estimated according to methods by (GUO & GIFFORD, 2002).

2.2.3 Soil analyses

Soil samples meant for C and nutrient analysis were initially oven-dried at 30 ∘C for 48 h. Dried samples were sieved with a 2 mm sieve to remove coarse particles and plant roots. Sieved samples were milled and stored in a desiccator before analyses. Soil samples were analyzed for C and total soil N (*t*N) with the C–N analyzer (Vario MAX CN; Germany), using aspirin (50 mg; N – 9.7 %; C – 34.0 %) and a standard soil sample (1 g; N – 1.2 %; C – 1.4 %) after every ten tests of soil samples to aid in the calibration of the equipment. Bulk density was estimated after oven-drying at 105 °C. It was assumed that soil samples did not contain inorganic C because pH values were less than seven and because no liming or any other amendment has been carried out during the past 50 years; therefore, total C was considered SOC. Additional randomly performed HCl tests confirmed this assumption.

Soil pH was determined according to methods by (WIESMEIER *et al.*, 2012). Soil pH was measured directly with a pH meter (microprocessor pH/ION meter; PMX 3000; WTW) after adding 0.0125 M $CaCl_2$ solution to each sample in the ratio of 1:2.5 (soil / $CaCl_2$ solution). Plant available P (*a*P) and exchangeable K (*e*K) were extracted from 1 g air-dried fine soil (< 2 mm) using the Bray 2 solution, with the reagents being 0.1 M HCl and 0.03 M NH_4F (BRAY & KURTZ, 1945). *e*K was determined using flame photometry, and *a*P was measured calorimetrically at 882 nm (MILLER & ARAI, 2016) after a reaction with ammonium molybdate and the development of the molybdenum (Mo) blue colour (within 30 min).

2.2.4 Plant sampling and proanthocyanidin analyses

Forage samples for CTs determination were harvested during the late annual growth stage. Sampling included leaves and leaf stalks from dicots, which consisted of legume herbs and legume and non-legume tree and shrub species (Appendix 2.1). After cutting, samples were immediately cooled on ice before being freeze-dried, milled with a ball mill and stored in a freezer at −28 ∘C until further analyses. Condensed tannins (CTs; synonym – proanthocyanidins), which consisted of extractable CTs (ECTs), protein-bound tannins (PCTs) and fibre-bound tannins (FCTs), were determined according to methods prescribed by (TERRILL *et al.*, 1992). Despite newer analytical techniques being available, TERRILL *et al.* (1992) provide the benefit of separating again between protein- and fibre-bound tannins, and hence, this was considered the most suitable method. Extractable condensed tannins (ECTs) were extracted from 20 mg plant samples using an acetone and water mixture (80:20, v⁄v), vortexed for 5 min and shaken on a plenary shaker (280 min−1) at 4 ∘C overnight. The samples were then centrifuged for 10 min (14 000 rpm) and decanted into 2 mL Eppendorf tubes. Residues were extracted again, using the same set-up, using the plenary shaker now shaking for three h, and centrifuged, and the supernatant was decanted on top of the first extract. Acetone was evaporated in each case in an Eppendorf concentrator plus (Eppendorf, Hamburg, Germany) at room temperature for 90 min. Extracts were frozen overnight and, after that, freeze-dried for 24 h. A total of 1 mL of ultra-performance liquid chromatography (UPLC)-grade water was added to the freeze-dried extracts, vortexed and filtered with a polytetrafluoroethylene (PTFE) filter (0.2 µm).

Protein-bound condensed tannins (PCTs) were extracted twice from the residues by adding 10 g L−1 sodium dodecyl sulfate (SDS) and 50 g L^{-1} 2-mercaptoethanol in 10 mM Tris-Cl adjusted to a pH of 8, vortexed for 5 min and then placed in a continually boiling water bath for 60 min and cooled on ice to room temperature. The mixture was then centrifuged for 10 min, and the supernatant was decanted into 2 mL Eppendorf tubes in triplicates. For analysis, 960 µL of n-Butanol and HCl (95:5, v⁄v) solution was added to 240 µL of the extract of either ECTs or PCTs, vortexed for 5 min and heated in an oven at 90 ◦C for 90 min; after that, it was cooled on ice and transferred to a spectrophotometer (Libra S22; Biochrom) and analyzed at 550 nm to determine the CT concentrations. For the fibre-bound condensed tannin (FCT) concentration in the samples, 1200 µL of BuOH⁄HCl (95:5, v⁄v) and 120 µL SDS was added to the residues from the extracts, vortexed for 5 min, centrifuged for 10 min and heated in an oven for 90 min at 90 ◦C before being cooled on ice to room temperature, centrifuged for 1 min and then measured in the spectrophotometer at 550 nm. Total CT (tCT) was calculated as the sum of ECTs, PCTs and FCTs for each candidate species. Annual *t*CT was estimated by multiplying *t*CT with the mean biomass yield of each functional group. The data set and a brief description of the site, materials and methods adopted to generate data are available in NYAMEASEM *et al.* (2020).

2.2.5 Calculations and statistics

Due to the absence of data regarding the farmland's initial SOC stocks, we estimated per cent changes in soil C stocks using a pseudo baseline. This pseudo baseline is the natural grassland, representing the SOC and nutrient stocks that all other sampled areas would have had they not been converted to farmland and agricultural research plots. We believe this assumption to be valid as all sampled areas were part of the same natural grassland until being converted, around the same time, to their current land use. With environmental conditions and atmospheric deposition being identical for all sites due to their proximity, the variation among plots is assumed to result from the land-use change only. Thus, the natural grassland represents the trajectory for soil conditions as they would occur across the entire grassland region without land-use change and, thus, represents the baseline against which all sites were tested.

We performed a one-way ANOVA using generalised linear models to analyse the effect of the different land-use types on SOC and soil properties. Also, we tested the effects of plant functional groups (legume trees/shrubs, legume herbs and non-legume trees/shrubs) and plant parts (leaves and leaf stalks) on the polyphenol content of the forages in a two-way ANOVA, using linear mixed effect modelling, with species as a random factor. *P* values were estimated based on the type II sum of squares (SSs) in the one-way ANOVA and type III SSs in the two-way ANOVA due to the dissimilarity of the sample sizes (FOX & WEISBERG, 2019). In cases where P values were significant, Tukey's post hoc tests, using the “lsmeans” function of the “multcomp” package (BRETZ *et al.*, 2011), were performed to permit pairwise comparisons of the means. Before the ANOVA, data were checked for normality and the homogeneity of variance. In abnormality or heteroscedasticity cases, data were log-transformed or corrected using “White-adjusted heteroscedasticity-corrected standard errors” from the “car” package of R. Where data normality or the equality of variances were not confirmed even after log transformation, a non-parametric test (Kruskal–Wallis) was used, followed by Dunnett's post hoc tests to permit pairwise comparisons of means of the unequal sample sizes (ZAR, 2010). Full correlations, a bivariate (linear and exponential) and a full hierarchical model were fitted to the data sets to establish relationships between the measured variables and SOC and identify the main effects and interaction terms as SOC predictors. Akaike's information criterion, with a small sample bias adjustment (BURNHAM *et al.*, 2011)(AIC_C; AKAIKE, 1985;), was estimated and used to identify the best-fit model. All the statistics were performed using R (R DEVELOPMENT CORE TEAM, 2019).

2.3 Results

2.3.1 Land use change, soil C stocks and soil chemical properties

The different land-use types had varying effects on soil C stocks in the upper soil layer (0–30 cm), with group means ranging from 19.9 to 36.8 Mg C ha^{-1} and a mean and standard deviation of 32.9 ± 10.2 Mg ha^{-1}. Whereas SOC stocks did not differ between the agricultural soils and native grassland soils ($p>0.05$), grazed–seeded grassland soils had 86 % and 77 % less ($p<0.01$) SOC density relative to cut–use trees/shrubs and grasses, respectively (Figure 2.3a). On the other hand, SOC was 36 % higher for cut-use forage systems among the cut–use grasses relative to legume herbs ($p<0.05$).

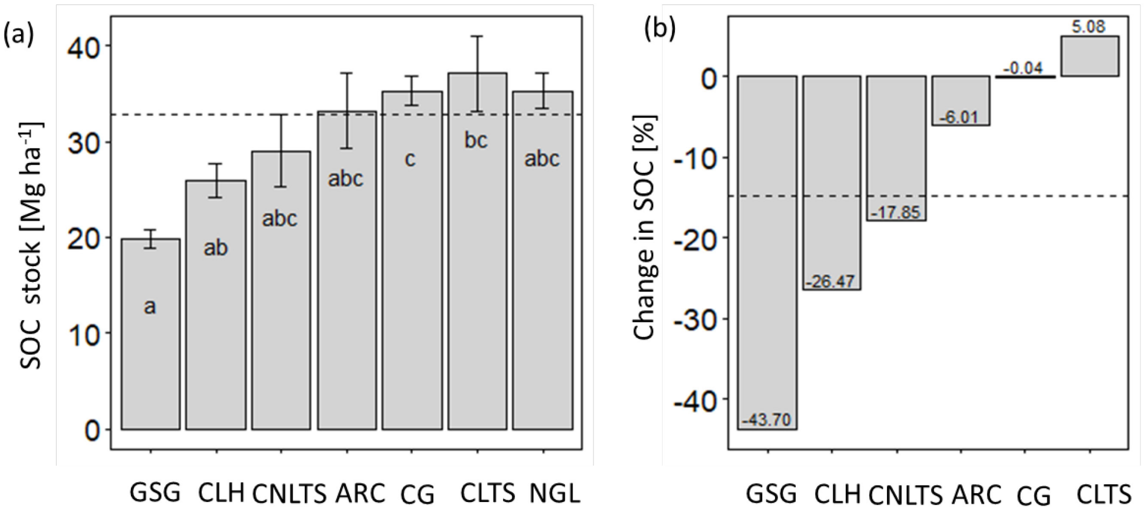


Figure 2.3. Barplots (with error bars) of SOC stocks (A) and per cent changes in SOC stocks (B) due to native grassland conversion under different land-use scenarios. [Different lowercase letters indicate significant differences between the land-use types ($p<0.05$; se = 1.13); CLTS= cut-use legume trees/shrubs; NGL= native grassland; CG= cut-use grasses; ARC= arable crops; CNLTS=Cut-use non-legume trees/shrubs; CLH= cut-use legume herbs; GSG = grazed-seeded grasslands; broken horizontal line indicate the means].

Using the native grassland as a pseudo baseline, we observed SOC stock changes ranging from −44 % to 5 %, with a mean loss of −15 %, and the most considerable negative change occurring in grazed–seeded grassland soils (Figure 2.3b). However, there appeared to be near-zero to positive changes in cut–use grass and legume trees/shrub soils. Among the cut–use fodder groups, SOC losses in soils of cut–use legume herbs were 26 % greater relative to cut–use grass soils. The mean (± sd) OM, *t*N, *a*P and *e*K concentrations observed for the site were 1.32 ± 0.41 %, 0.057 ± 0.02 %, 10.4 ± 11.9 mg kg^{-1} soil and 68.1 ± 36.7 mg kg^{-1} soil, respectively. Whereas *t*N, *a*P and *e*K concentrations among the native grassland and agricultural soils ($p>0.05$), cut–use grasses soils contained 37 % more N compared to legume herb soils (Table 2.2).

Table 2.2. Soil organic carbon, soil macro-nutrients and other chemical properties of soils under different long-term management

Soil chemical trait	Native grassland	Arable crops	Grazed-seeded grassland	Cut-use forage production				*P*-value	SEM
				Grass	Legume herb	Legume trees/shrub	Non-legume tree/shrubs		
tN, Mg ha^{-1}	2.52^{ab}	2.48^{ab}	1.75^{ab}	2.66^{b}	1.94^{a}	2.55^{ab}	1.93^{ab}	0.0196	0.09
AvP, kg ha^{-1}	89.6^{ab}	185^{b}	15.0^{a}	37.8^{a}	28.1^{a}	112^{b}	73.6^{ab}	0.0001	8.23
K, Mg ha^{-1}	6.96^{ab}	16.6^{b}	3.34^{a}	7.42^{ab}	4.45^{a}	7.43^{ab}	4.80^{ab}	0.0028	0.52
C: N ratio	14.4^{b}	13.6^{ab}	11.3^{a}	13.5^{ab}	13.6^{ab}	14.6^{ab}	15.2^{b}	0.0489	0.22
Soil pH	5.69^{bc}	5.80^{c}	4.71^{ab}	5.35^{bc}	4.79^{a}	5.27^{abc}	5.42^{abc}	0.0037	0.05

tN= soil total nitrogen; Av. P= plant-available phosphorus; K= soil potassium. ^{abc}Mean values in the same horizontal row with different letters are significantly different ($p< 0.05$); SEM, standard error of the mean

Arable crop soils contained 5, 7 and 12 times more *a*P density compared with cut–use grass, herbs and seeded–grazed soils ($p<0.001$), respectively (Table 2.2), while *e*K density in arable crop soils was 5 and 4 times higher ($p<0.05$) compared with seeded–grazed and legume herb soils, respectively (Table 2.2). CN ratio was 27 % higher in native grassland soils ($p<0.05$) compared with seeded–grazed and was lower ($p<0.05$) for grazed–seeded grasslands compared with non-legume trees/shrubs. It appears that C: N ratio values were not different among the forage species ($p>0.05$). Soil pH ranged from moderately acidic to neutral and was affected by the land use types, was more acidic in the cut–use herb soils relative to the grasses, arable crop and native grassland soils, and was more acid for seeded–grazed fields relative to arable crop soils ($p<0.05$).

Table 2.3. Condensed tannin (CT) profile and CT yield from three plant functional groups growing at the experimental site

Functional group	Condensed tannins (g kg^{-1} DM)				*t*CT yield* (kg ha^{-1} yr-1)
	ECTs	PCTs	FCTs	*t*CTs	
Legume herbs	2.63^{a}	2.60^{a}	2.78^{a}	8.01^{a}	32.7^{a}
Legume trees/shrubs	10.6^{b}	8.52^{b}	6.07^{b}	25.1^{b}	217^{b}
Non-legume trees/ shrubs	5.83^{ab}	3.24^{ab}	4.79^{ab}	13.9^{b}	120^{b}
P-value	<0.001	<0.0001	<0.0001	<0.0001	<0.0001
SEM	1.06	0.72	0.65	1.99	16.9

ECTs= extractable condensed tannins; PCTs= protein-bound condensed tannins; FCTs= fibre-bound condensed tannins; *t*CTs= total condensed tannins (ECT+PCT+FCT); By plant functional groups, values in a same column with different letters are significantly different. *Annual *t*CT yield was estimated using the mean annual dry matter yields

2.3.2 Plant secondary metabolites in cut–use forages

The CT distribution in the forages was affected by plant functional groupings ($p<0.001$) but not by the part of plant analysed or by plant group–plant part interaction terms ($p>0.05$). Accordingly, the functional group effect on CT distribution in the forages is presented in Table 2.3. ECTs, PCTs and FCTs in the legume herbs were 4, 3 and 2 times, respectively, lower ($p<0.001$) compared with legume trees/shrubs but not with non-legume trees/shrubs ($p>0.05$). However, *t*CT per dry matter (DM) and total annual *t*CT yield were 2–3 times and 4–6 times, respectively, higher ($p<0.001$) in both trees/shrubs compared with the herbs (Table 2.3).

2.3.3 Relationships between soil organic carbon and soil/plant chemical parameters

Pairwise correlation between the measured variables suggested significant ($p<0.01$) positive associations between SOC and all the soil chemical properties (Table 2.4), with the strongest association between SOC and *t*N (r – 0.86). Although the CT variables correlated positively with SOC and *t*N, the associations were weak and insignificant, except for annual *t*CT yield (Table 2.4). Whereas *t*N, *e*K and C:N ratio related exponentially with SOC, *a*P and soil pH related linearly with SOC (Figure 2.4a–e). Generally, higher *t*N,*a*P and soil pH appeared to be associated with higher SOC stocks; however, there appears to be a weaker association between SOC and *t*N at higher soil N concentrations. Whereas the response of SOC to *t*N was similar for non-legume trees/shrubs, grasses and legume herbs (mean slope=– 10.5), it was about 36 % higher compared with legume trees/shrubs (slope = 14); see Figure 2.5.

Table 2.4. Pearson correlation coefficients showing the relationships between soil and plant chemical parameter

	Soil pH	ECTs	PBCTs	FBCTs	*t*CTs	Ann*t*CT	*a*P	*e*K	SOC	*t*N
CN ratio	0.01	0.24^{*}	0.15	0.21^{*}	0.24^{*}	0.33^{*}	0.18	0.14	0.29^{**}	-0.22^{*}
Soil pH		0.02	-0.05	-0.11	-0.04	0.28^{*}	0.24^{*}	0.30^{**}	0.53^{***}	0.55^{***}
ECTs			0.59^{***}	0.66^{***}	0.90^{***}	0.84^{***}	0.22^{*}	-0.04	0.14	-0.01
PBCTs				0.66^{***}	0.84^{***}	0.78^{***}	0.22^{*}	-0.04	0.12	0.02
FBCTs					0.87^{***}	0.63^{***}	0.05	-0.08	0.01	-0.13
*t*CTs						0.93^{***}	0.20^{*}	-0.06	0.11	-0.04
Ann*t*CT							0.31^{*}	0.39^{**}	0.47^{***}	0.29^{*}
*a*P								0.27^{**}	0.29^{**}	0.19^{*}
*e*K									0.62^{***}	0.55^{***}
SOC										0.86^{***}

ECTs= extractable condensed tannins; PCTs= protein-bound condensed tannins; FCTs= fibre-bound condensed tannins; *t*CTs= total condensed tannins (ECT+PCT+FCT); Ann*t*CT = annual *t*CT yield; Significance levels *<0.05, **<0.01, ***<0.001.

Higher soil K density was associated with higher SOC stocks, but amounts beyond 16 Mg ha^{-1} appeared to depress SOC accumulation, whilst the relationship between SOC and C: N ratio appeared positive only beyond a ratio of 15 (Figure 2.4c–d). SOC related exponentially with *t*CT yield, with generally higher biomass CTs associating positively with SOC at increased rates beyond 200 kg ha^{-1} (Figure 2.6). A full regression model predicting SOC density using the measured soil and plant chemical variables as predictors showed significant effects of *t*N, *a*P, *e*K and *t*CT and *a*P × pH and *t*N × *a*P × *e*K interaction terms (p<0.05) in the model, with *t*N having the most significant effect (Table 2.5).

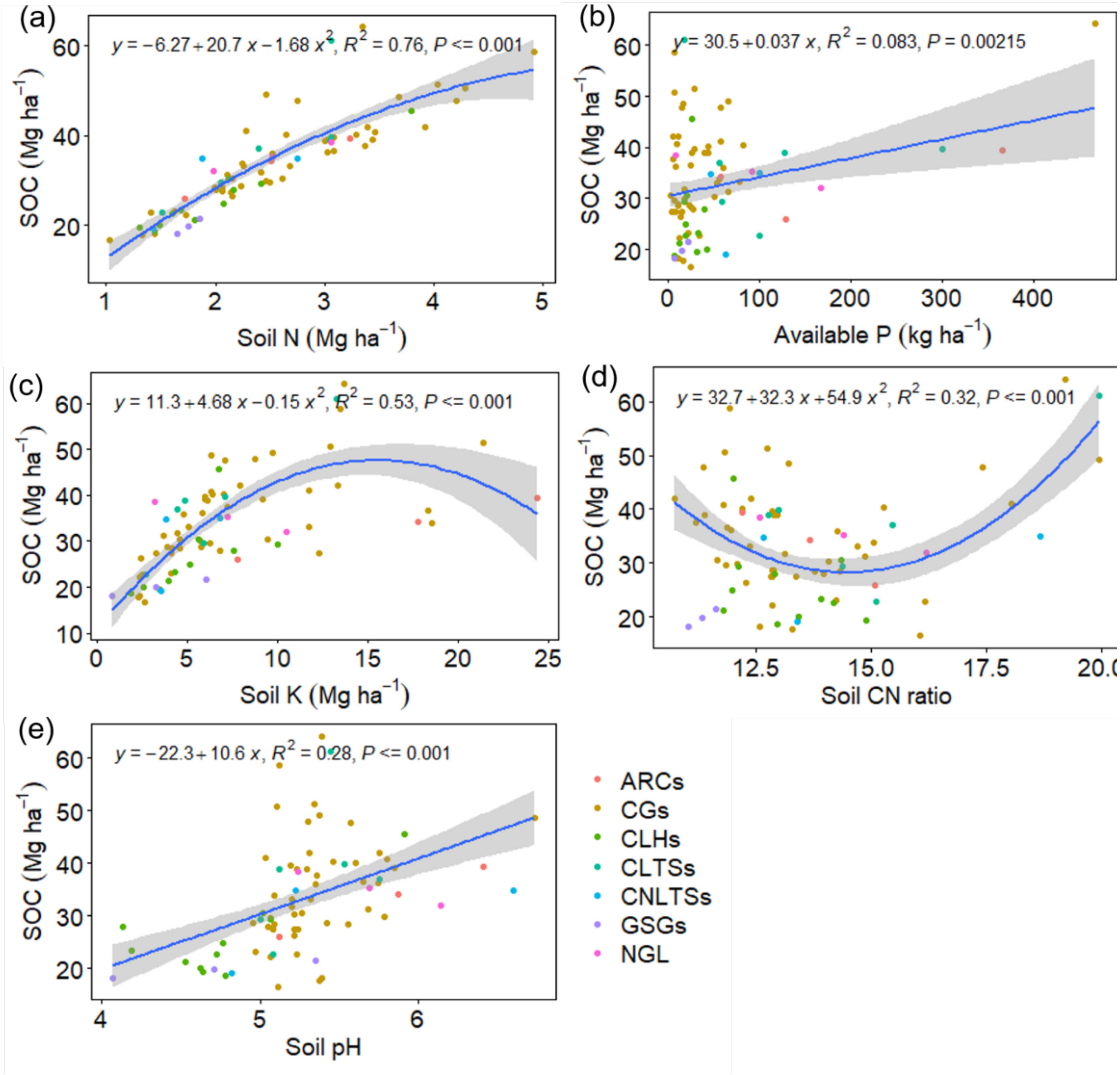


Figure 2.4: Bivariate regression curves showing the relationship between SOC and the measured soil variables. The grey band shows the 95 % confidence interval; CLTs = cut-use legume trees/shrubs; NGL= native grassland; CGs = cut-use grasses; ARCs= arable crops; CNLTs = cut-use non-legume trees/shrubs; CLHs= cut-use legume herbs; GSGs = grazed-seeded grasslands

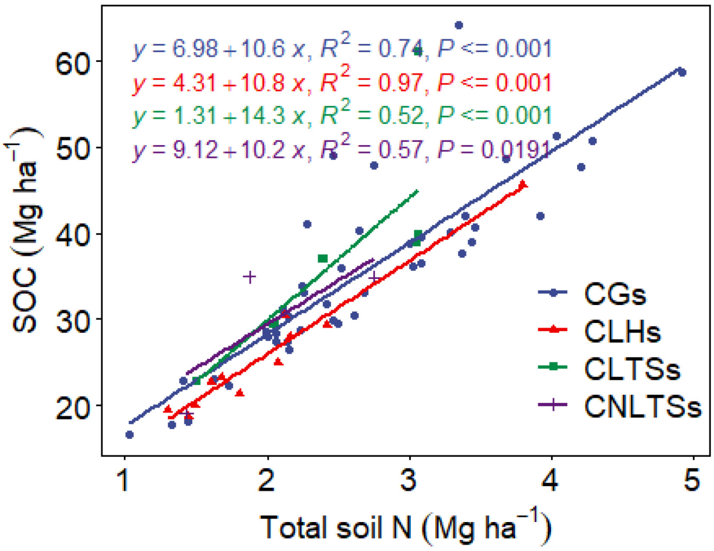


Figure 2.5: Relationships between SOC and soil total N as influenced by plant functional groups. CLTs = cut-use legume trees/shrubs; CGs = cut-use grasses; CNLTs=cut-use non-legume trees/shrubs; CLHs = cut-use legume herbs

Table 2.5. Results of full regression model to explain the status of soil organic carbon density at the study site ($R^2 = 0.848$, Adjusted $R^2 = 0.853$, $AIC_C = 698.60$*)

Source	Degrees of freedom	Type III sum of squares	*F* value	Pr(>F)
*t*N	1	2694	144.9	0.000
*a*P	1	73.53	3.955	0.049
*e*K	1	414.1	22.27	0.000
pH	1	34.99	1.882	0.173
*t*CT	1	230.6	12.40	0.001
*t*N x *a*P	1	28.62	1.539	0.218
*t*N x *e*K	1	3.316	0.178	0.674
*a*P x *e*K	1	202.1	10.87	0.001
*t*N x pH	1	56.44	3.036	0.085
*a*P x pH	1	116.7	6.277	0.014
*e*K x pH	1	9.120	0.491	0.485
*t*N x *a*P x *e*K	1	73.85	3.972	0.049
*t*N x *a*P x pH	1	13.26	0.713	0.400
*t*N x *e*K x pH	1	11.33	0.609	0.437
*a*P x *e*K x pH	1	11.76	0.632	0.428
*t*N x *a*P x *e*K x pH	1	33.69	1.812	0.181
Residuals	100	1859		

*Akaike's Information Criterion (AIC) (AKAIKE, 1985; BURNHAM & ANDERSON, 1992) with the small-sample bias adjustment ($AIC_C = n*[\ln (SSE/n)] + 2K + [(2K*(K + 1))/(n-K-1)]$) (HURVICH & TSAI, 1995; BURNHAM & ANDERSON, 2002); *t*N = total N, *t*CT= total condensed tannins, *a*P = available P.

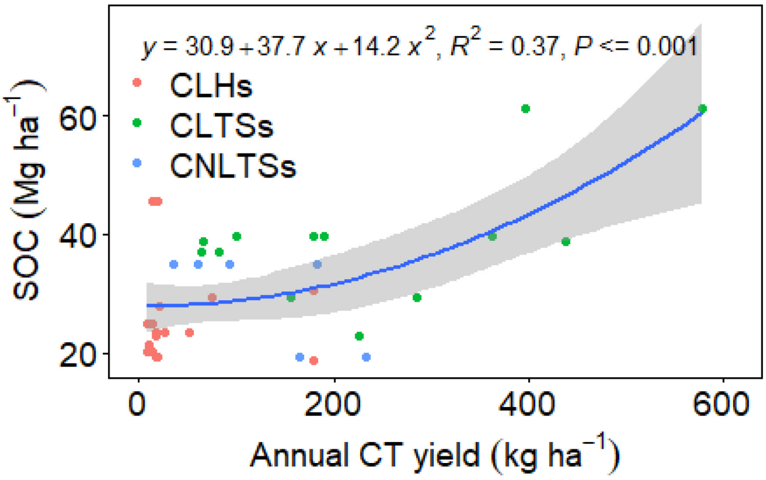


Figure 2.6: A graph showing the stoichiometric relationship between SOC and leaf biomass CT. The grey band shows the 95 % confidence interval; CLTs = cut-use legume trees/shrubs; CNLTs = cut-use non-legume trees/shrubs; CLHs= cut-use legume herbs

2.4 Discussion

2.4.1 Land use change and carbon stocks

We observed a wide range of SOC stocks (17–64 Mg SOC ha^{-1}) across the study site's land-use types. The observed SOC is consistent with previously reported ranges of 10–50 Mg SOC ha^{-1} for vegetation gradients varying from the Sudanese–Sahelian savannah to a subtropical forest (BESSAH *et al.*, 2016; SAIZ *et al.*, 2012). Similar to previous reports (BESSAH *et al.*, 2016; OLSON, 2013), the use of grassland for agriculture resulted in both positive and negative responses by soil C dynamics. (TAN *et al.*, 2009) projected changes of −4 % to −23 % in soil C stocks of cultivated savannahs of Ghana on a 100-year scale, depending on the climate change scenarios. Hence, the 15 % loss of SOC in 50 years appears to be on the higher side. Nevertheless, there was an indication of C sequestration in the cut–use legume trees/shrub production.

Similarly, a previous study (SHANMUGAM *et al.*, 2018) reported a mean annual change of 0.67 ± 0.95 Mg C ha^{-1} for tropical mineral soils of secondary woodland or savannah converted to cultivated pasture or cropland. Therefore, our observations are not surprising as the type of land management adopted after converting native vegetation affects soil C cycling and determines the soil's ultimate C storage potential (STAHL *et al.*, 2017; 'T MANNETJE *et al.*, 2008). From our study, SOC stocks of arable crop farming did not differ from the native grassland. Arable crop production is known to deplete SOC due to the tillage effect, facilitating SOC cycling. Among the three arable crop fields considered in this study, only one of the fields occasionally adopted conventional tillage. However, crop production was on a rotational basis on that particular field and occurred only occasionally, i.e. tillage occurred only infrequently. Soils were manually minimum tilled on the other crop fields using simple farm implements such as hoes and sometimes herbicidal weed control. Thus, this is a much less invasive technique than regular ploughing (but a representative for many farms in that area), and hence, it appears that the net effect of the mixed management practices on the arable crop farms did not impact SOC stocks significantly.

The low SOC stock in grazed–seeded grasslands was slightly above half (20 vs 35 t SOC ha^{-1}) that of cut–use grasses and legume trees/shrubs, suggesting a utilization effect. However, the effect size of grazing on SOC stocks might depend on the interactions of several factors, including grazing intensity (MCSHERRY & RITCHIE, 2013). Overgrazing has been widely reported as responsible for the loss of SOC and decreased soil fertility in West Africa (SAIZ *et al.*, 2012; SCHÖNBACH *et al.*, 2011). Generally, higher grazing intensities decrease soil C and N by direct removal of above-ground herbaceous biomass, thus reducing the potential of CO_2 fixation in photosynthetic tissue and reducing below-ground C inputs through lower root production and higher root litter turnover (SEMMARTIN *et al.*, 2010). On the other hand, moderate grazing may increase tiller density and above-ground productivity, particularly in C_4-dominated grasslands (MCSHERRY & RITCHIE, 2013) compared to cut–use systems, thereby leading to higher C inputs because of additions from crop residues and leaf senescence, assuming appropriate supplies of abiotic factors such as irrigation and nutrient supply. Thus, reducing grazing intensity may help protect the above-ground biomass of grasslands and improve soil structure and enhanced the accumulation of organic C (XU *et al.*, 2018).

The effect of plant functional differences on SOC stocks was evident in this study. We observed higher C stocks in fodder grass fields (36 % higher; $p<0.01$) than legume herb fields. Earlier reports (SHANMUGAM *et al.*, 2018; ALONSO *et al.,* 2012) suggested that the species composition of grasslands influences OM input quantity and C sequestration in the soil. Dry matter productivity is an essential factor that influences the accumulation of soil C. Above-ground biomass productivity is higher for fodder trees/shrubs relative to that of grasses and lowest in herbaceous legumes (Table 2.1). SOC stocks under the cut–use forages appear to follow a similar trend (Table 2.2; Figure 2.3). The greater ability of legume trees/shrubs to produce relatively high amounts of biomass (ADJOLOHOUN *et al.*, 2008; BARNES & ADDO-KWARFO, 1996), maintain soil fertility (as shown in Table 2.2), control runoff-related soil erosion

(FRANZEL *et al.*, 2014) and maintain SOM partly explains the higher SOC stocks observed under legume trees/shrubs relative to legume herbs.

Grasslands containing more C_4 grass species were reported to store more SOC than grasslands with more C_3 and legume species (YANG *et al.*, 2019). The grass species considered in this study consisted mainly of C_4 grasses, whereas the legume herbs were mainly C_3 shallow-rooted plants. C_4 plants possess morphogenic and architectural traits that enhance their ability to outperform C_3 plants, particularly under harsh environmental conditions (LATTANZI, 2010). For example, they tend to develop a high leaf area index, enhancing their ability to capture light, N and water compared to their C_3 counterparts. Besides, C_4 plants have higher photosynthetic efficiency in water and N use, yielding higher quantum productivity (TAYLOR *et al.*, 2010) than C_3 plants. In addition, the inherently lower Rubisco concentration and the more lignified tissue in C_4 plants limit organic matter decomposition, thus producing more recalcitrant organic C (YANG *et al.,* 2019). Moreover, C_4 plants are reported to partition C towards roots in N limited situations; thus, they can fix soil C under N stress situations (SAGE & PEARCY, 1987; LONG, 1999). Another reason could be that C_4 plants have a higher concentration of amino acids and organic acids in their root exudates than C_3 plants (NABAIS *et al.,* 2011), which could protect SOC and N from microbial decomposition. Therefore, the relative differences in biomass characteristics could be responsible for the observed differences in SOC stocks under fodder grass and legume herbs fields.

2.4.2 Impact of differential land uses and plant types on soil quality

We observed a higher N concentration in the soil of cut–use grass fields than legume herb fields, although legumes are known to fix atmospheric N in soils. Indeed, in the semi-arid and savannah ecosystems of sub-Saharan Africa, legume herb and legume browse species are reported to fix 8–217 and 61–643 kg ha^{-1} yr^{-1} atmospheric N, respectively (HASSEN *et al.*, 2017). Accordingly, legume-based systems can produce high biomass yield even in the absence of N fertilizer application, showing a high N cycling efficiency (SCHMEER *et al.*, 2014) and generally increasing soil N concentration. However, several factors may affect N fixation in legumes, including environmental conditions, N uptake by plants and soil pH (NUTMAN, 1976; SAGE & PEARCY, 1987). In addition, pure grass stands may show a high fraction of C and N allocation below-ground (LOGES *et al.*, 2018), particularly when the functional traits equilibrium theory of the roots is considered (BROUWER, 1983) and the stands have amino-acid-rich roots (NABAIS *et al.*, 2011), leading, potentially, to a more excessive N and C accumulation in the soil. These attributes of grasses might explain why soils in grass plots had higher *t*N than the legume herbs. The fairly tight C: N ratio (14:1) observed for the agricultural systems reflects the stoichiometry of stable soil OM at the study site.

This study site has seen a general decline in soil fertility (*t*N and SOM), although there were marginal increases in available P and K compared with the values reported for the site by BARNES (1999). While P and K concentrations were moderate in arable crop soils, they were low in the other land use types (BATIONO *et al*., 2018; APAL AGRICULTURAL LABORATORY, 2020). The relatively high P and K values observed in the food crop fields may probably be due to their annual supply through fertilizer application (Table 2.1). Cut–use grasses and legume herbs appeared to deplete soil P, but this effect was not apparent for legume or non-legume trees and shrubs. This trend might be partly related to differences in the root architecture. The grasses and herbs are shallow-rooted and thus exploit nutrients from within the upper soil depth, while the trees/shrubs have roots below the 30 cm soil depth considered in this study and might be less able to exploit nutrients extensively from the upper layer of the soil. The relatively low nutrient concentrations observed in the grazed–seeded grasslands matches the low C stocks (Table 2.2). It is particularly striking that P and K are much lower in grazed grassland despite greater excreta deposition associated with grazing. Although we could not ascertain the stocking rate on the grazed–seeded pastures, it appears that there was frequent overgrazing of the pastures, which is a regular feature in this region, exposing the soils to nutrient loss via erosion (BATIONO *et al*., 2018).

The soils were generally acidic, a common characteristic of tropical soils (Jayne, 2015). Associated effects of the <5.5 pH observed in grazed–seeded grasslands, cut–use legume herbs and non-legume trees/shrubs soils might cause aluminium and manganese toxicities. Low pH could also cause molybdenum, calcium, magnesium or potassium deficiencies and perhaps reduced microbial activity (APAL AGRICULTURAL LABORATORY, 2020). Among the land use systems, cut–use legume herbs and seeded–grazed soils appeared more acidic relative to the others. Legume plants commonly form symbiotic associations with rhizobia and accumulate most of their N through symbiotic N fixation. During this process, legume plants take up more cations than anions and release more H^+ ions from roots to the soil, leading to low pH values in both the rhizosphere and bulk soil (YANG *et al*., 2016; ZHAO *et al*., 2009). This effect might differ between legume herbs and legume trees/shrubs due to their root architecture differences. Grazing fields are associated with high N and C returns from animal excreta, but C and N cycles might cause acidification in grazed fields; for example, nitrate leaching might increase the concentration of H ions, which might lead to decreased soil pH (RIDLEY *et al*., 1990).

2.4.3 Condensed tannin concentration in cut–use forages as affected by functional group differences

The total CT concentration of below 67 g kg^{-1} DM observed in this study is similar to values reported for tropical forages, with lower concentrations in legume herbs than for browse species (MUPANGWA *et al.,* 2000; SOTTIE *et al*., 2016). CTs may have both positive and negative effects on livestock, depending on their concentration in the plants. The nutritional implications of CTs are particularly vital in the case of tropical shrubs and tree species, as high CTs are known to impair protein availability (JAYANEGARA *et al*., 2019). Previous authors have reported higher values of more than 70 g CT kg^{-1} DM for some tropical shrubs and trees (ROSALES, 1999; JACKSON *et al*., 1996; PEREIRA *et al*., 2018) which invariably limits their use as fodder for ruminant livestock.

The proportion of total CTs allocated to ECTs was similar to the range of 12 %–44 % reported by MUPANGWA *et al.* (2000) but lower than the 70 %–95 % reported for some tropical browse species (JACKSON *et al*., 1996). Compared with other tropical forage species, the *t*CT shares of below 7 % indicate comparably low tannin concentrations, which indicates their suitability as potential feeds for livestock (JACKSON *et al*., 1996; MUPANGWA *et al*., 2000). However, further analyses on forage quality parameters and tannin composition and other plant secondary metabolites might be required to give reliable estimates of their digestibility value.

2.4.4 Relationships between SOC and soil and plant chemical properties

Our correlation and regression analyses (Figure 2.4; Tables 2.4 and 2.5) indicated significant relationships between the soil chemical properties and SOC. Similar to reports of (STAHL *et al*., 2017), we found a close association between C and N. Nevertheless, in the literature, the relationship between N and SOC is ambiguous, with evidence of different responses of soil CO_2 fluxes to N levels in soils (YAN *et al*., 2016), including increases (WANG *et al*., 2015), decreases (JIANG *et al*., 2010) and no significant differences (LI *et al*., 2012). The N cycle is strongly interconnected with the C cycle as N is required for cell growth and division and, therefore, for biomass accumulation (and hence of C). Whereas (SIX *et al*., 2002) attributed the positive relationship between soil C and N to protection by both macro-and micro-aggregates against mineralization, (WALDROP *et al*., 2004) explained that greater N availability reduces the decomposition rate of SOC by regulating the production and activity of microbial extracellular enzymes. Indeed, when N becomes available in N-limited soils, photosynthetic reaction increases, thus enhancing SOC storage, but when amounts of N are low, this may limit the CO_2 fertilization effect (LAL, 2018). Accordingly, (TAN *et al.*, 2009) observed that increasing N application to about 30–60 kg ha^{-1} yr^{-1} could positively change the soil C stocks of Ghana's cultivated grasslands. In the present study, the multiple linear regression model (Table 2.5) showed that *t*N had the most significant effect on SOC density and with a greater effect in size in legume trees/shrub species compared with the legume herbs, non-legume trees/shrubs and grasses (Figure 2.5).

Interaction effects involving N, P and K and pH and K were significantly associated with SOC stocks ($p<0.05$), suggesting that N's effect on SOC sequestration might depend on P, K and the soil pH. The associative effect of N and P on SOC density was also reported in an earlier study (BRADFORD *et al.*, 2008), where P and N additions led to a more significant C sequestration in soils. In a simulated study (LI *et al.*, 2014), a combination of N and P fertilizers increased SOC storage and reduced microbial activity and C mineralization compared to adding N or P fertilizer alone or no fertilizer. In contrast, (GRAHAM *et al.*, 2014) reported a significant release of CO_2 when soil N increased. In the current study, if the relative proportions of soil nutrients impact SOC storage and soils deficient in P are less able to use N efficiently, this might increase N losses by gaseous emissions and leaching and contribute to the depletion of SOC pools.

According to linear mixed-effect regression models (Table 2.5), the theoretical basis suggested tCT concentration in above-ground biomass as one of the potential factors that might affect SOC storage at the site (Table 2.5). This observation partly confirms our hypothesis that above-ground CT concentration might be influential in C storage. Although CT concentrations in the soils were not considered in this study, we speculate that the higher CT content in legume trees/shrubs (Table 2.3) might have slowed the decomposition rate of soil OM (DONG *et al.*, 2016) to promote SOC accumulation (ADAMCZYK *et al.*, 2016, 2017; CHOMEL *et al.*, 2016; HALVORSON *et al.*, 2011; KAGIYA *et al.*, 2019; KRAUS *et al.*, 2003; TAMURA & THARAYIL, 2014). By extension, the higher potential of the trees/shrubs to produce more mass and more CTs per unit area might explain the higher SOC stocks under trees/shrubs relative to those for the herbs. However, the bivariate regression analysis showed that positive associations between *t*CT and SOC only occurred at concentrations above 200 kg ha^{-1} yr^{-1} (45 mg kg^{-1} DM). Although tropical herbs contained relatively higher CTs than their grass counterparts, the CT concentration might not be potent enough to impact SOC storage, as shown in this study (*t*CT = 10 ± 8; <45 mg kg^{-1} DM). Although tropical herbs contained higher amounts of CTs than their grass counterparts, SOC density was lower in soils under the herbs compared with grasses. Even when the CT concentration in the biomass is low, it might be that, in the rhizosphere, it is higher due to accumulation. Below-ground biotic and abiotic conditions, including the presence of some fungi (e.g. *Basidiomycetes* and *Ascomycetes*) and bacteria species (e.g. *Pseudomonas*), temperature, pH, oxygen and substrate availability, are factors that affect the degradation of phenolic compounds in soils (MIN *et al.*, 2015). Although the effect of pH on polyphenol degradation is not consistent, under laboratory conditions low soil pH (<5) was shown to be optimal for extracellular enzymes involved in degrading phenolic compounds in soils (MIN *et al.*, 2015). In our study, soil pH was lower in soils under the legume herbs compared with the grasses (Table 2.2). This low soil pH might have facilitated a faster degradation of the potentially accumulated CTs and mitigated the assumed effect of CTs on SOC.

We observed significant positive associations between annual CT yield and all the measured soil parameters. A previous study (MUDAU *et al.*, 2007) demonstrated that, regardless of the season, application of N, P and K fertilizers increased the total polyphenols in bush tea (*Athrixia phylicoides* L.), suggesting that N, P and K limitation might affect the synthesis of CTs by plants and for SOC storage. Furthermore, the low nutrient density in the soils might partly explain the lower concentration of CTs in the forages compared to earlier reports on similar species (ROSALES, 1999; JACKSON *et al.*, 1996; PEREIRA *et al.*, 2018). Thus, the proportion of extractable CTs and bound CTs, even within species, might change with plant maturity and environmental conditions (JACKSON *et al.*, 1996) and might influence the forage's feeding value. Consequently, increased soil fertility might have some implications for CT concentration in forages and, thus, for livestock feeding and SOC storage.

We speculate that, at the farm level, where climatic and soil properties are relatively uniform, differences in land use might influence the quantity and quality of organic matter added to the soil. These variables might have induced plant-soil feedbacks to regulate soil C cycling at the micro-level, causing local variations in C stocks (CHEN *et al.*, 2018; POST *et al.*, 1982; SAIZ *et al.*, 2012).

2.4.5 Implications of the study

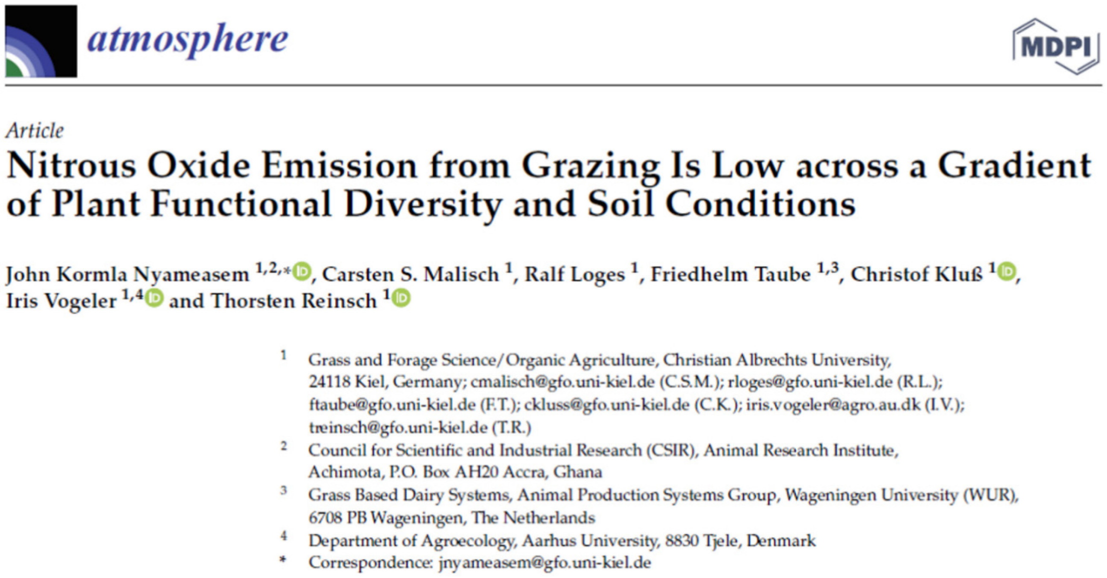
This study has shown that soil nutrients have implications for SOC input and removal and, by extension, for primary plant productivity and ecosystem functioning (MARQUES *et al.,* 2016;

POST *et al*., 2012). However, most Ghanaian soils are inherently infertile (BATIONO *et al.,* 2018) as a result of leaching, soil erosion by rainfall (0.6–0.9 t ha^{-1}) and the fact that nutrients removed by crop harvest are not replaced by the corresponding amount of plant nutrients (BONSU, 1979; BATIONO *et al*., 2018). Consequently, the low concentration of soil nutrients across the fields may partly explain the low SOC stocks observed for soils in the study site. These fragile soils would require sustainable agricultural land-use systems to ensure higher below-ground net primary productivity (NPP) for increased C stocks.

The forage legume herbs showed the lowest yields (Table 2.1), indicating low bioactivity compared with grasses, whereas the legume shrubs had the highest yields and, thus, captured a lot of N from the biomass. Besides, soils under the shrubs contained similar *t*N compared with grasses, suggesting that the tested forage legume herbs were not suitable species regarding biomass yield for forage and soil carbon sequestration. Therefore, legume shrubs should be preferred, as they provide more forage and ecosystem services. Invariably, our findings further extend the benefits of cut–use trees/shrubs as they already contribute to food security, incomes and livelihoods in Africa. Furthermore, these plants are deep-rooted, resistant to drought and maintain high protein levels during the dry season when high-quality feed is scarce (WAMBUGU *et al*., 2011).

2.5 Conclusions

We tested the hypothesis that the conversion of native grasslands can cause considerable losses in sub-Saharan Africa's soil C stocks. Although SOC stocks in the agricultural soils did not differ statistically from the native grassland soils, most of the land transformations resulted in declining SOC stocks, questioning the potential of such farms to achieve the 4 ‰ target. However, the observed wide range of SOC stocks suggests an enormous potential for SOC storage in the area. Our findings further extend the benefits of cut–use trees/shrubs as they already contribute to food security, incomes and livelihoods in Africa. The inclusion of cut–use shrubs/trees or their integration with grass fodder production could be researched further as they show the potential to store more C. All the measured soil chemical properties correlated positively and significantly with SOC, endorsing the fact that soil chemicals are essential drivers of NPP in ecosystems. Hence a boost in productivity is also most likely to result in a boost in SOC. Condensed tannins were positively associated with SOC and could be explored further to harness their potential to extend SOC residence time in soils. Inappropriate pasture management regarding livestock stocking rate and choices relating to grazing or forage harvest, species selection and nutrient management could affect plant CTs' transformation efficiency to soil CTs. We hypothesize that sustainable soil management practices, coupled with the adoption of CT-rich forages, might improve the SOC storage capacity of livestock production systems in this ecological region of Ghana.



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Nitrous Oxide Emission from Grazing Is Low across a Gradient of Plant Functional Diversity and Soil Conditions

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CHAPTER 3

Nitrous oxide emissions from grazing are low across a gradient of plant functional diversity and soil conditions

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This chapter explored the potential of legume-based mixed pastures to mitigate nitrous oxide emission under grazing pressure. This chapter is an original work of the author of this thesis, from the experimental planning, the field and analytical work to the article's writing.

Abstract

Nitrous oxide (N_2O) emissions from pastures can vary significantly depending on soil and environmental conditions, nitrogen (N) input, as well as the plant's ability to take up the N. We tested the hypothesis that legume-based N sources are characterized by significantly lower emission factors than mineral N based dairy systems. Therefore, this study monitored N_2O emissions for a minimum of 100 days and up to two growing seasons across a gradient of plant species diversity. Emissions were measured from both grazed pastures and a controlled application of urine and dung using the static chamber method. About 90% of the simulated experiments' accumulated N_2O emissions occurred during the first 60–75 days. The average accumulated N_2O emissions were 0.11, 0.87, 0.99, and 0.21 kg ha^{-1} for control, dung, urine patches, and grazed pastures, respectively. The N uptake efficiency at the excreta patch scale was about 70% for both dung and urine. The highest N_2O-N emission factor was less than half compared with the IPCC default (0.3 vs 0.77), suggesting an overestimation of N_2O-N emissions from organically managed pastures in temperate climates. Plant diversity showed no significant effect on N_2O emission. However, functional groups were significant ($p < 0.05$). We concluded that legume-containing pasture systems without a fertilizer addition generally appear capable of utilizing nitrogen inputs from excreta patches efficiently, resulting in low N_2O emissions.

Keywords: plant diversity; nitrous oxide emission; grass-clover; rotational grazing; organic N fertilization

3.1. Introduction

Agriculture is susceptible to climate change, as global food systems are threatened by an increased risk of intra- and inter-annual yield variability due to the less predictable growing conditions (MYERS *et al.*, 2017). In addition, it is a contributor to climate change by greenhouse gas (GHG) emissions. Among the GHGs, nitrous oxide (N_2O) is about 265 times stronger in terms of the global warming potential compared with CO_2 (IPCC, 2014), as it destroys the ozone layer in the troposphere (PORTMANN *et al.*, 2012). The N_2O in the atmosphere was increasing linearly at a rate of 0.26% per year (TIAN *et al.*, 2015) and was estimated for 2007–2016 to be about 17.0 Tg N $year^{-1}$ (TIAN *et al.*, 2020). Agricultural soils are the foremost significant contributor to the global anthropogenic N_2O emissions (about 3.8 Tg N $year^{-1}$) due to the mineral or organic fertilizer application (TIAN *et al.*, 2020). Similarly, pastures emit N_2O, as grazing animals return high loads of N (200 to 2000 kg ha^{-1} $year^{-1}$) in urine and dung patches (SELBIE *et al.*, 2015), which exceed the use capacity of grasslands. The excess N results in losses via leaching, volatilization, and N_2O emissions (LUO *et al.*, 2018). Accordingly, pastures account for 86% of the net global N_2O emissions from grasslands, and almost 2/3 of these result from animal excretion (DANGAL *et al.*, 2019).

Generally, N_2O emissions from pastures are dependent on soil type, climate, N input, the sward composition, and grazing management (LUO *et al.*, 2018; REINSCH *et al.*, 2018). Particularly, soil moisture and ambient temperature influence N_2O emission significantly by modulating the microbial activity and transport of gases in soil (MCAULIFFE *et al.*, 2020; VAN DER WEERDEN *et al.*, 2014). Furthermore, soil moisture affects nitrification and denitrification processes (DI *et al.*, 2014; VAN DER WEERDEN *et al.*, 2014). The N fertilizer application plays an important role (OENEMA & SAPEK, 2000). External N additions might increase N losses from the ecosystems (BURKE *et al.*, 2002) due to the potential N oversupply and the relatively fast N mineralization beyond plants' uptake rate. The external N fertilization use is restricted in organic production systems. Consequently, N_2O emissions are elevated in conventional production systems using high N–fertilizers per area (NÚÑEZ *et al.*, 2007) compared with legume-containing grassland swards (SCHMEER *et al.*, 2014).

Generally, the use of diverse pastures containing forage legumes is a promising strategy for the sustainable intensification of livestock production in northwest Europe (SCHAUB *et al.*, 2020). Multispecies pastures of different functional traits (particularly grasses and legumes) have the potential to provide ecological services, including the provision of primary productivity with fewer nutrient inputs due to the improved niche complementarity and inter-species facilitation, resulting in “overyielding” effects compared to monocultures (NYFELER *et al.*, 2009). Furthermore, legume-derived nitrogen has the additional advantage of providing a continuous and more synchronized N flow to meet the plants’ requirements, thus reducing N_2O emission intensity (FUCHS *et al.*, 2020). Furthermore, the additional inclusion of herb species in the pastures can further increase above- and belowground biomass by increasing the share of deep-rooted non-leguminous species (CONG *et al.*, 2017). Besides, herbs, such as *Plantago lanceolata*, may reduce N_2O emission losses from grazed grasslands (DE KLEIN *et al.*, 2019; LUO *et al.*, 2018). Some plant species, such as *Plantago lanceolata*, may affect the soil’s N cycling processes through root exudation of secondary plant metabolites, including biological nitrification inhibitors (BOWATTE *et al.*, 2018; DE KLEIN *et al.*, 2019). However, the soil-environment interaction effect might affect the degree to which plants mediate N cycling (PODOLYAN *et al.*, 2020), and the potential of these species in mixtures is not yet fully understood (FUCHS *et al.*, 2020). Therefore, there is high uncertainty regarding the effects of the botanical composition of pastures on the GHG balance (FRANZLUEBBERS, 2019).

In accordance, recent studies (CHADWICK *et al.*, 2018; KROL *et al.*, 2016; MARSDEN *et al.*, 2016; SIMON *et al.*, 2018; THOMAS *et al.*, 2017; VOGLMEIER *et al.*, 2019) have reported low N_2O-N emission factors (EFs) for urine and dung patches in improved grazing management systems, compelling the Intergovernmental Panel on Climate Change (IPCC) to revise the IPCC (2006) default emission factor from 2 to 0.77% for wet temperate climates (IPCC, 2019). This study assessed the applicability of the revised default N_2O EF (IPCC, 2019) to an organic dairy farm located on sandy loam soil in northern Germany. Accordingly, we measured the N_2O emission from three mixed grasslands differing in diversity under actual and simulated grazing conditions. To simulate the highest possible N_2O emissions, we also measured N_2O emissions from an irrigated binary mixture. We explored the potential drivers of N_2O emission at the site to better understand N dynamics to inform the development of site-specific mitigation strategies. Accordingly, we hypothesized that (i) grassland diversity affects N_2O emission from pastures under grazing stress, (ii) botanical, environmental factors determine N_2O emission from soils, and (iii) N_2O EF for cow excreta deposited on irrigated pastures is lower for systems with the legume as a sole N source compared with the IPCC default.

3.2. Materials and methods

3.2.1. Site characteristics

The experiments were conducted from 2018 to early 2020 at the organic research farm Lindhof (54°27′ N, 9°57′ E; elevation 27 m above sea level) in northern Germany. The soils at the site belong to the Eutric Luvisol soil class (FAO, 2014), with a mean pH of 6.4. The soil texture comprised 13% clay, 26% silt, and 61% sand in the 0–30 cm soil depth, soil bulk density of 1.56 g cm^{-3}, 1.45 mg kg^{-1} of NH_4-N, 1.13 mg kg^{-1} of NO_3-N, total N of 0.12%, and organic carbon of 2.2% (Appendix B, Table B1). The study was located in a region of high grazing intensity due to the well-distributed precipitation across the year. The long-term (1981–2019) mean temperature and annual precipitation of the site are 9.7 °C and 859 mm, respectively.

The grasslands consisted of a binary (*prwc)*, a ternary (*prwrc*), and a multispecies (*prwrch*)-sward, with two to three different functional groups (grasses, legumes, non-legume herbs). As previously reported (LOGES *et al*., 2020), the seed mixtures used were a binary mixture of 24 kg ha^{-1} perennial ryegrass (*Lolium perenne,* PR) + 4 kg ha^{-1} white clover (*Trifolium repens*, WC) (PR + WC = prwc), a ternary mixture of 24 kg ha^{-1} PR+ 2 kg ha^{-1} WC + 6 kg ha^{-1} red clover (*Trifolium pratense*, RC) (PR + WC + RC = *prwrc*), and a multispecies mixture of 16 kg ha^{-1} PR+ 1.5 kg ha^{-1} WC + 3 kg ha^{-1} RC that additionally contained herbs (1 kg ha^{-1} ribwort plantain (*Plantago lanceolata*) + 2 kg ha^{-1} of each chicory (*Cichorium intybus*), sheep's burnet (*Sanguisorba minor*), caraway (*Carum carvi*), and 5 kg ha^{-1} birdsfoot trefoil (*Lotus corniculatus*) (PR + WC + RC + herbs = *prwrch*).

Previously, the site was used for arable cropping in a 5-year crop rotation until 1993, when it was converted to "Bio-land" (prohibiting the use of chemical fertilizers and pesticides) and subsequently managed as grassland (grass-clover mixtures). The grassland was managed in a mixed system (1–2 silage cuts followed by 3–4 grazing cycles by cattle). Two years before the start of the experiment, the site was ploughed to establish the pastures. They were managed organically and without any external nutrient input. Therefore, legumes were the primary source of nitrogen supply.

3.2.2. Environmental conditions

Weather parameters, including daily precipitation and soil temperature, were obtained from a meteorological station located within a 70 m radius of the experimental area. The daily maximum and minimum air temperature, daily precipitation, and water-filled pore space (WFPS) in the soils during the studies are shown in Figure 3.1. The mean annual temperature at the site was similar for 2018 and 2019 (10.3 °C) but 6% higher than the long-term average (LTA, 1981–2019). The mean soil temperature was relatively higher in summer than in spring or autumn in 2018 and 2019. In both experimental years, soil temperature following excreta deposition was slightly below 10 °C in spring but rose quickly and was between 18–20 °C during summer excreta application and decreased to about 15 °C during autumn excreta applications (Figure 3.1).

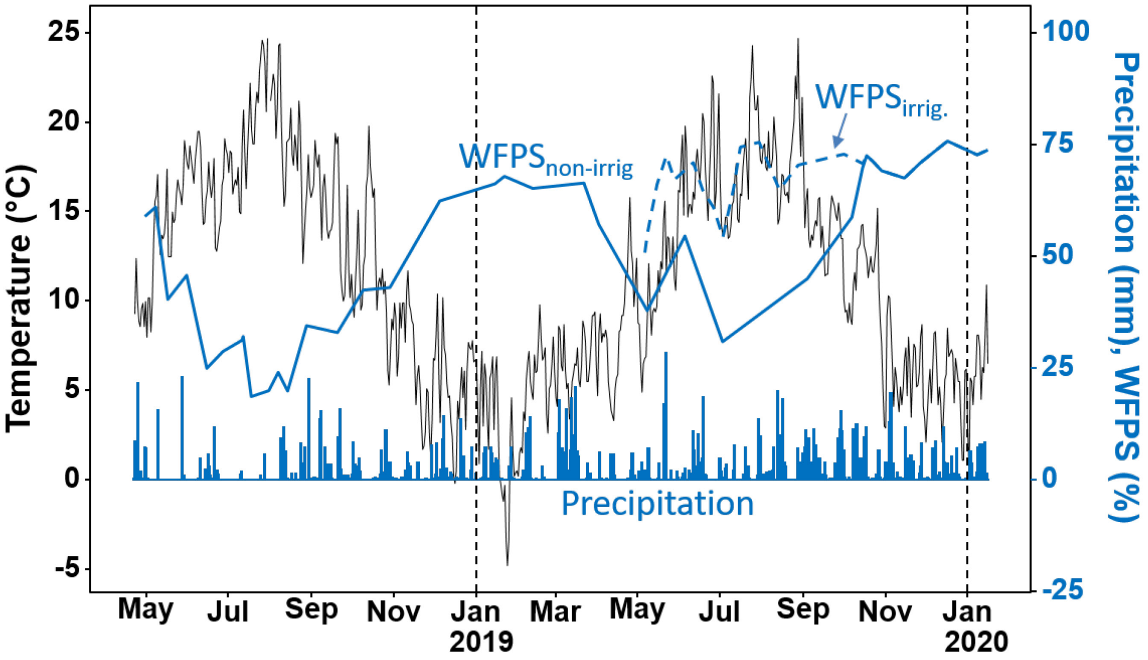

Figure 3.1. Weather parameters and water-filled pore space (WFPS) during the experimental period. Blue (broken) lines demarcate the experimental years; non-irrig: Non-irrigated; irrig.: Irrigated.

Precipitation was 25% lower (647.4 vs 859.2 mm) and 7% higher (922.4 vs 859.2 mm) for 2018 and 2019, respectively, compared with the LTA. Whereas the spring of 2018 and 2019 had 1.6–2 times higher precipitation relative to the LTA, their summers and autumn of 2018 had reduced (20–40%) precipitation than the LTA. WFPS ranged between 21.3–71.4% (mean ± SD; 43.5 ± 16.0) in the simulated grazing plots following the excreta deposition in 2018. Accordingly, urine and dung patches experienced different environmental conditions after each excreta application event (Figure 3.1). Therefore, WFPS ranged between 58.2–86.8% (mean ± SD = 77.8 ± 7.7%) across seasons following the excreta application (Figure 3.1). Consequently, the mean WFPS was about 1.6 times higher for the irrigated pastures than non-irrigated ones.

3.2.3 Experiments

Three experiments were conducted to test the hypotheses set out for this study. *Experiment one* was set up to quantify N_2O emission from the three diverse pastures (*prwc, prwrc*, and *prwrch*) under grazing stress. Measurements spanned the period of April 2018 to January 2020. The second experiment was a simulated grazing trial which also started in 2018. Therefore, cow urine and dung with a known amount of N were applied to the three diverse pastures to establish their response to N_2O emission and to calculate N_2O emission factors (EF).

Table 3.1. Summary of the experimental designs and measurements

Experimental design	Measurements
Experiment 1: Grassland diversity affects N_2O emission from pastures under grazing stress	
Static chambers were established on the three diverse pastures (*prwc*, *prwrc* and *prwrch*); in a randomized complete block design, laid out in three replicate blocks (18 experimental units in all); each replicate plot measured 12 x 9 m; Jersey dairy cows rotationally grazed on a three–five-week cycle depending on the pastures' growth rate	Gas sampling was done at least once a week to measure N_2O; gas sampling started from April 2018 to January 2020, weather parameters were monitored;
Experiment 2: Botanical, environmental factors determine N_2O emission from soils	
Static chambers arranged in a split-split plot design; the diverse grasslands as main plots, excreta treatments as split-plots, and season as split-split plots, laid out in three replicated blocks (27 experimental units per each set-up); urine and dung applied once in spring, summer and autumn; each application was measured for one year; duplicate plots for soil sampling.	Gas sampling started immediately after excreta application; was done at least once a week for one year, except in December where only three samples were taken; weather parameters, soil mineral N concentration, biomass dry matter and N yields, species composition, water-filled pore space (WFPS) were monitored.
Experiment 3: N_2O EF for cow excreta deposited on irrigated pastures is lower for systems with legume as sole N source compared with IPCC default.	
Static chambers were arranged in a split-plot design, using the binary swards; with excreta treatments as main plots and the season of excreta application as split-plot, all laid out randomly in one block with four replicates (12 experimental units per each set-up); duplicate plots were used for soil sampling;	Gas sampling was done at least once a week for 100 days; weather parameters, mineral N concentration, biomass dry matter and WFPS were monitored; sampling duration was 100 days.

The third experiment was also a simulated grazing trial established in April 2019 and was meant to establish N_2O EF under good soil moisture conditions (which the study site is known for), as 2018 was unusually dry. For this, only the binary mixture (*prwc*) was used. Finally, the pasture was irrigated using sprinkler method to simulate rain at least a day before gas sampling each week, depending on the WFPS (i.e., when WFPS fell below 60%), to maintain adequate soil moisture for pasture growth and denitrification. The summary of the experiments, including the specific hypotheses, study design, and measurements, are shown in Table 3.1.

3.2.3.1 Urine and dung collection, handling and application

We collected dung and urine from dairy cows kept under confinement systems (fed conserved fodder and concentrates) to ensure an almost constant N content of dung and urine throughout the year. Urine and dung specimens were collected immediately after morning milking (~05:00 h). Urine samples were taken midstream after manual stimulation of the vulva, while faeces samples were collected as the animal defecated. Faeces and urine were homogenized and stored immediately under refrigeration (<4 °C) and were applied within seven days. When removed from the refrigerator, the urine and dung were allowed to attain ambient temperature before application.

Homogenized and representative quantities of fresh urine and dung were applied once per measurement period (1 year or 100 days) in spring, summer, and autumn to avoid introducing new effects due to differences in excreta volume or mass (CARDOSO *et al.*, 2018). Urine was applied at a constant rate of 8.8 L m^{-2} from a height of 40 cm and was expected to cover the entire collar by percolation (CICHOTA *et al.*, 2018), whereas each dung patch of 2.2 kg covered an approximate area of 0.041 m^2. The timing of excreta application, N concentrations, and total amount of N applied per ha are shown in Table 3.2.

Table 3.2. Cow excreta chemical characteristics and application rates.

Parameters	**Experiment 2**						**Experiment 3**					
	Spring		**Summer**		**Autumn**		**Spring**		**Summer**		**Autumn**	
	U	**D**	**U**	**D**	**U**	**D**	**U**	**D**	**U**	**D**	**U**	**D**
N (%)	0.44	0.49	0.52	0.42	0.46	0.39	0.54	0.45	0.51	0.36	0.43	0.41
C:N	2.7	12.2	2.2	14.2	2.5	15.3	2.5	14.5	3.4	14.5	4.0	12.5
Application rate (kg m^{-2}) *	2.5	2.2	2.5	2.2	2.5	2.2	2.5	2.2	2.5	2.2	2.5	2.2
N loading rate (kg ha^{-1})	458	449	542	385	479	358	563	413	529	326	451	380
Date of application	30.04.2018		11.07.2018		08.10.2018		06.05.2019		16.07.2019		09.10.2019	

U: Urine; D: Dung; * excreta was applied only once per measurement period.

3.2.3.2 N_2O sampling

We sampled gas fluxes using the closed static chamber method (Hutchinson and Mosier, 1981). This method consisted of polyvinyl chloride (PVC) collar (h = 15 cm, d = 60 cm) installed to a depth of 10 cm into the soil on each replicate plot. The chamber was made of PVC, 30 and 40 cm high for grazed pastures and simulated grazing, respectively, with an internal diameter of 60 cm. In each sampling session, the chamber was deployed onto the plastic collar between 10:00 and 12:00 h (REINSCH *et al.*, 2018), with the chamber's collar sealed with a rubber belt.

In addition, one air sample was taken with a 30-mL polypropylene syringe from the chamber-top at 0, 20, 40, and 60 min after closure. The syringe's air sample was transferred to 12 mL pre-evacuated glass vials (Labco, High Wycombe, UK) and stored for a maximum of 2 weeks before analyses.

3.2.3.3 Soil and biomass sampling

Each micro plot for gas measurement had a duplication adjacently placed to evaluate WFPS, ammonium (NH_4), and nitrate (NO_3) concentrations in the top 25 cm of soil. At least six soil samples were taken in this duplicated plot within the first two months following excreta application and at least once in the subsequent months, using a core sampler. The soil samples were stored immediately at −17 °C until further processing. Biomass was harvested from the rings and micro-plots in synchrony with the grazing cycle in *Experiment one*. Harvesting was done by cutting all forage within each subplot at a 5 cm height from the soil surface. Total fresh matter (FM) weight of each plot’s biomass was recorded immediately after harvesting. After determining the fresh weight, two sub-samples (80–100 g) were taken, one for dry matter determination and nutrient analyses, while the second sub-sample was sorted into three functional groups (grass, legumes, and non-legume herbs).

3.2.4. Chemical analyses

Urine and dung samples were analyzed in duplicates by dry combustion using a C/N analyzer (Vario Max CN, Elementar Analysensysteme, Hanau, Germany) to determine total carbon and nitrogen concentrations, helium as a purge and carrier gas. Two sub-samples of dung were dried at 60 °C to determine the dry mass content. Biomass sub-samples were oven-dried to a constant weight at 55 °C to determine their dry matter. The dried samples were then milled through a 1-mm sieve (Cyclotech mill, Foss analytical, Hilleröd, Denmark) and later used to determine N concentration by near-infrared reflectance spectroscopy (NIRS) using a NIR-System 5000 monochromator (Foss Analytical, Hillerød, Denmark). Soil samples were extracted with 0.01 M $CaCl_2$; NO_3 and NH_4 concentrations were determined photometrically, using a dual-channel continuous flow analyzer (Skalar Analytical Instrument, Breda, the Netherlands). We used 50–80 g of each soil sample to determine the gravimetric moisture by oven-drying the fresh samples at 105 °C to a constant weight. This index was used to correct soil moisture and to calculate WFPS. N_2O concentration in the sampled gas was analyzed by gas chromatography (model 7890a, Agilent Technology Inc., Santa Clara, CA, USA) equipped with a ^{63}Ni electron-capture detector. Helium was used as the carrier gas and argon-methane as the make-up gas. Three certified gas standards (300, 620, 1510 ppb) were measured regularly to determine the analytical precision (standard deviation of 10 repeated measurements <3 ppb). The gas chromatograph procedure used a detector temperature of 320 °C, a column temperature of 40 °C, and an injector temperature of 200 °C.

3.2.5. Calculations

We estimated biologically fixed N (BFN) using a model (Eq. 1; HØGH-JENSEN *et al.*, 2004),

$$\mathrm{BNF} = DM_{legume} \text{ x N\% x } P_{fix} \text{ x } (1 + P_{root+stubble} + P_{transsoil} + P_{transanimal} + P_{immobile}) \qquad \text{(Eq. 1)}$$

where DM_{legume} is the accumulated amount of legume shoot dry matter above the standard defoliation height; N% is the concentration of N in the dry matter of the legume (kg kg^{-1}); P_{fix} is the fixed N_2 as the proportion of total N in the shoot dry matter of the legume; $P_{root+stubble}$ is the fixed N_2 in the root and stubble as the proportion of totally fixed shoot N at the end of the growing period; $P_{transsoil}$ is the belowground transfer of fixed legume N_2 located in the grass mixtures as the proportion of total fixed shoot N at the end of the growing period; $P_{immobile}$ is the fixed N_2 immobilized in an organic soil pool at the end of the growing period as a proportion of fixed shoot N; $P_{transanimal}$ is the above-ground transfer (by grazing animals) of fixed legume N_2 located in the grass in mixtures as the proportion of total fixed shoot N at the end of the growing period. To parameterize the model for N%, P_{fix}, $P_{root+stubble}$, $P_{transoil}$, and $P_{immobile}$, we used 0.0375, 0.845, 0.25, 0.15, and 0.315, respectively for swards with white and red clover, and 0.04, 0.80, 0.25, 0.25, and 0.38, for white clover only swards (HØGH-JENSEN *et al.*, 2004), $P_{transanimal}$ was set to zero. We considered the N applied, BFN, and annual deposition by precipitation (12.5 kg N ha^{-1}; (MINISTRY OF ENERGY, AGRICULTURE, THE ENVIRONMENT, NATURE AND DIGITALIZATION OF SCHLESWIG HOLSTEIN, 2008) as N input and N yield in harvested biomass as N output. Patch residual N (PRN) was calculated as the difference between N input and output (PARKIN & VENTEREA, 2010). This parameter indicated the available N liable to denitrification and nitrification losses, leaching, ammonia emissions, and immobilization.

Volumetric water content (VWC) was calculated as the product of gravimetric water and soil bulk density (BD) (Appendix Table B1); and WFPS was calculated (Equation (1)) after considering VWC, the BD, and a particle density (PD) of 2.65 g cm^{-3}:

$$\mathrm{WFPS} = \frac{\mathrm{VWC}}{1-\mathrm{BD/PD}} \text{ x } 100\% \qquad \text{(Eq. 2)}$$

We calculated NO_3 and NH_4 intensities in the 0–25 cm soil depth for 100 days sampling duration by submitting the daily NO_3 and NH_4 data to linear interpolation. NO_3 or NH_4 intensity is an integrated measure of the accumulation of mineral N in the soil over time (SIMON *et al.*, 2018). N_2O-N fluxes were calculated for each treatment and replicate by linear regression between measured N_2O-N concentrations and time (PARKIN & VENTEREA, 2010). Hourly (Equation (3)), daily, cumulative N_2O emissions, as well as emission factors, were estimated according to methods described by KROL *et al.* (2016). The hourly N_2O-N flux (µg m^{-2} h^{-1}) was estimated by taking account of the slope (Δc/Δt) of the line that describes the concentration increase of N_2O inside the chamber during 60 min deployment; the temperature, pressure (assumed as 1 atm), and internal volume of the chamber (V); and the area of micro plot delimited by the metal base (A); the molar weight (M) of N in N_2O; and the ideal gas constant (R):

$$F(hourly) = \left(\frac{\Delta c}{\Delta t}\right) x \frac{MxP}{RxT} x \left(\frac{V}{A}\right) \qquad (Eq. 3)$$

Daily N_2O-N emission (g ha^{-1} d^{-1}) was calculated by multiplying the hourly fluxes by 24 h. Cumulative emission of N_2O-N in each season (kg ha^{-1}) was estimated by integrating the fluxes over each season's monitoring period (area under the curve). N_2O-N emission factor (EF) was calculated using the IPCC (2006) recommended method (Equation (4)), which only considered the amount of N applied. However, as all swards contained legumes, we also estimated N_2O-N EF using (Equation (5)) based on total N input (modelled BFN + N applied):

$$EF = \frac{N_2ON_{Treatment} - N_2ON_{Control}}{N\ applied}\ x\ 100\% \qquad (Eq. 4)$$

$$EF_1 = \frac{N_2ON_{Treatment/Control}}{Total\ N\ input}\ x\ 100\% \qquad (Eq. 5)$$

3.2.6. Statistics

We performed all statistical procedures and produced all graphs using R software (R DEVELOPMENT CORE TEAM, 2019). For *Experiment one*, the effect of grassland diversity on N_2O emission from grazed pastures was tested using a generalized linear model with the diverse grasslands as a fixed effect and the block as a random factor. For Experiments 2 and 3, N_2O emission, soil, and biomass variables were analyzed using a mixed-effect model with grassland, the season of excreta application and excreta type or control as fixed factors and blocks and replicates as random factors, except for 2019 data where the season of excreta application and excreta type were considered as fixed factors and replicates as random factors. As described, the treatment factors were used to analyze all the variables in a full factorial arrangement. In each case, the adequacy of the model was assessed by examining the appropriateness of residual plots. Variables were log-transformed where necessary before the analysis to ensure a normal distribution of residuals. We separated the effective means at $p < 0.05$ with Tukey's post hoc tests, using the "lsmeans" function of the "multcomp" package (BRETZ *et al.*, 2016). Regression tests (linear and quadratic) were performed to establish relationships between N_2O emission/EFs and the measured/estimated variables. The best-fitted models were selected based on their Akaike information criterion (AIC) with a small sample second-order bias correction (BURNHAM *et al.*, 2011).

3.3. Results

3.3.1 Plant yields, botanical composition and soil residual N

Grassland diversity significantly affected all the measured biomass traits ($p < 0.05$), except for the herbs (Table 3.3). Thus, whereas grass proportions in the swards decreased with increasing species richness, legume proportions were highest, intermediate and lowest for the ternary, multispecies and binary pastures, respectively. Consequently, BFN was highest, intermediate, and lowest for *prwrc, prwrch*, and *prwc*, respectively. In addition, the dry matter yield and herbage N were lower for binary swards than ternary or multispecies swards.

Urine and dung application significantly affected ($p<0.05$) most measured parameters but not dry matter yield or herb proportions (Table 3.3). Grass proportions and herbage N were highest, intermediate, and lowest for urine, dung, and control plots. However, legume proportions and the resulting BFN were lowest, intermediate, and highest for urine, dung, and control plots, respectively.

Seasonal differences in excreta application significantly affected legume proportions, dry matter yield, herbage N and BFN (Table 3.3). Whereas legume proportions only differed between spring and summer excreta treatments, dry matter yield and herbage N differed significantly between the three seasonal excreta applications, being highest, intermediate, and lowest for spring, summer, and autumn, respectively. In addition, BFN was higher for spring treatments compared with summer or autumn.

Grassland diversity, the season of excreta application and excreta type significantly affected ($p < 0.05$) N input, N uptake, and PRN (Table 3.3). N input was highest, intermediate, and lowest for *prwrc, prwrch*, and *prwc*, respectively. However, N uptake was lower for the binary swards than the ternary or multispecies swards, while PRN was only higher for *prwrc* than *prwc* or *prwrch*. Similar to the legume proportion and resulting BFN, N input was highest, intermediate, and lowest for urine, dung, and control plots. However, N uptake was higher for urine-treated plots than the control plots, while the resulting PRN was lower for the control than urine or dung treated swards. N input and PRN differed significantly ($p < 0.05$) between the three seasonal treatments, being highest, intermediate, and lowest for spring, summer, and autumn, respectively, but N uptake was higher in autumn compared with summer or spring.

Table 3.3. Mean values of the measured parameters showing the main effects of grassland diversity (grassland), excreta type (treatment), and timing of excreta deposition (season).

Parameters	Grassland			Treatment			Season			SEM
	prwc	*prwrc*	*prwrch*	Cont	Dng	Urn	Spg	Sum	Aut	
Experiment 2—Non-irrigated simulated grazing										
Grass (%)	74^{c}	55^{b}	47^{a}	48^{a}	60^{b}	67^{c}	57	61	58	1.77
Legume (%)	18^{a}	42^{c}	33^{b}	41^{c}	30^{b}	22^{a}	34^{b}	29^{a}	30^{ab}	1.62
Herb (%)	-	-	18	4	7	7	6	6	7	1.14
Unsown (%)	8^{b}	3^{a}	2^{a}	6	3	3	3	5	5	0.65
DM yield (Mg ha^{-1})	9.8^{a}	11.7^{b}	12.4^{b}	11.8	10.7	11.3	12.7^{c}	11.2^{b}	9.9^{a}	0.26
Herbage N (g N kg DM^{-1})	35.4^{a}	37.3^{b}	36.7^{b}	33.2^{a}	36.7^{b}	39.5^{c}	31.2^{a}	34.7^{b}	43.4^{c}	0.74
$^{\beta}$BFN (kg ha^{-1} year^{-1})	106^{a}	279^{c}	241^{b}	294^{c}	187^{b}	146^{a}	263^{b}	190^{a}	174^{a}	13.8
N input (kg N ha^{-1} year^{-1})	416^{a}	589^{c}	550^{b}	307^{a}	597^{b}	651^{c}	578^{c}	512^{b}	465^{a}	21.0
N uptake (kg N ha^{-1} year^{-1})	344^{a}	432^{b}	447^{b}	389^{a}	394^{a}	440^{b}	397^{a}	391^{a}	435^{b}	10.6
PRN (kg N ha^{-1} year^{-1})	67^{a}	152^{b}	98^{a}	-87^{a}	198^{b}	206^{b}	176^{c}	116^{b}	26^{a}	18.9
NO_3 (mg N kg^{-1} dry soil)	3.8	3.3	3.5	1.4^{a}	3.5^{b}	5.7^{c}	2.2^{a}	3.1^{a}	5.4^{b}	0.37
NH_4 (mg N kg^{-1} dry soil)	3.5	2.9	3.2	1.5^{a}	2.9^{b}	5.3^{c}	2.8	3.9	2.9	0.26
$^{\alpha}N_2O$ (kg N ha^{-1} year^{-1})	0.61	0.67	0.68	0.11^{a}	0.87^{b}	0.99^{b}	1.01^{c}	0.59^{b}	0.36^{a}	0.07
N_2O (kg N ha^{-1} 100 d^{-1})	0.46	0.57	0.53	0.03^{a}	0.71^{b}	0.83^{b}	0.92^{b}	0.48^{a}	0.17^{a}	0.07
g N_2O-N kg^{-1} N uptake year^{-1}	1.83	1.73	1.67	0.29^{a}	2.38^{b}	2.56^{b}	2.88^{c}	1.55^{b}	0.80^{a}	0.21
N_2O-N EF (%)	0.17	0.19	0.19	-	0.19	0.18	0.30^{c}	0.16^{b}	0.09^{a}	0.02
N_2O-N EF_1 (%)	0.13	0.10	0.11	0.04^{a}	0.14^{b}	0.16^{b}	0.16^{b}	0.11^{a}	0.08^{a}	0.01
Experiment 3—Irrigated simulated grazing										
NO_3 (mg kg^{-1} dry soil)	4.64	-	-	1.2^{a}	4.7^{b}	8.1^{c}	2.2^{a}	3.2^{a}	8.5^{b}	0.77
NH_4 (mg kg^{-1} dry soil)	4.80	-	-	1.8^{a}	3.5^{b}	9.1^{c}	4.6	5.0	4.8	0.65
$^{\alpha}N_2O$ (kg N ha^{-1} 100 d^{-1})	0.81	-	-	0.05^{a}	0.81^{b}	1.59^{c}	0.99	0.78	0.68	0.15
N_2O-N EF (%)	0.25	-	-	-	0.21^{a}	0.29^{b}	0.24	0.28	0.23	0.04

See Appendix B, Table B2 for the corresponding F- and *p*-values. Means with different letters are different at $p < 0.05$; SEM: Standard error of the pooled mean across grasslands, treatments, and seasons; β Estimated parameter. β BFN: Biologically fixed nitrogen; DM: dry matter; PRN: Patch residual N (PRN = N uptake − N input); N_2O-N EF1 was calculated as % of N input (BFN + applied N) emitted as N_2O-N; N_2O-N EF was calculated as % of applied N emitted as N_2O-N. * Accumulated for one year; α accumulated for 100 days. *prwc*: Perennial ryegrass and white clover swards; *prwrc*: Perennial ryegrass, white- and red clover swards; *prwrch*: Perennial ryegrass, white- and red clover + herbs. Cont=control; Dng=dung; Urn=urine; Spg=spring; Sum=summer; Aut=autumn

3.3.2 N_2O emissions

3.3.2.1. N_2O emissions from grazed pastures with a gradient in diversity

Daily N_2O-N fluxes from the grazed grasslands (Figure 3.2) ranged from 0.04–3.24 mg m^{-2} d^{-1} (mean ± SD = 0.31 ± 0.33). The 8-month (May–December) mean (SD) flux was slightly higher in 2019 (0.35 ± 0.46) compared with 2018 (0.29 ± 0.23). Grassland diversity had no apparent impact on the daily N_2O-N fluxes, except for 2019, where the highest peak was observed from the *prwrc* swards (Figure 3.2). The peaks usually followed precipitation events and appeared to occur a few days after a grazing activity.

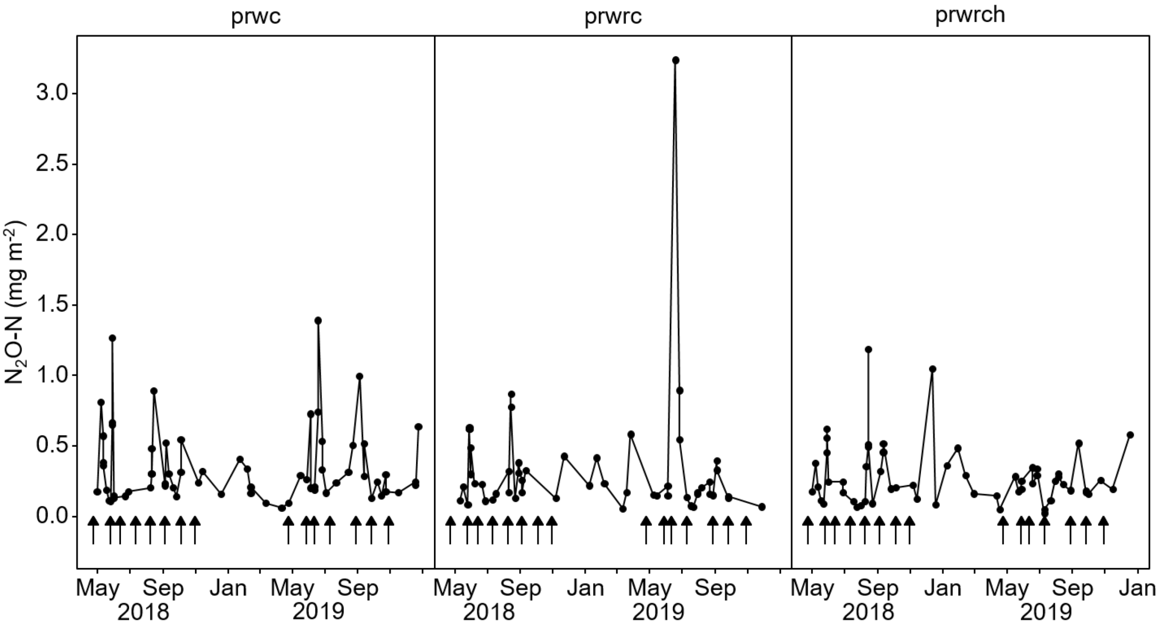

Figure 3.2. Daily N_2O-N emissions from diverse grasslands grazed by dairy cows. *prwc* = perennial ryegrass and white clover swards; *prwrc*= perennial ryegrass, white- and red clover swards; *prwrch*= perennial ryegrass, white- and red clover + herbs. The arrows indicate grazing events.

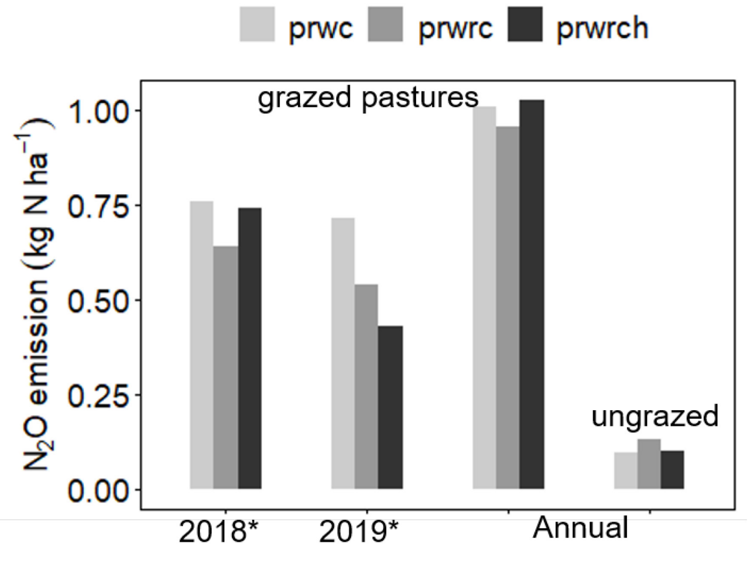

Figure 3.3: Mean values of the N_2O-N emission from diverse pastures grazed by dairy cows in a 3–5 week rotational cycle. *accumulated for eight months (May–December); the annual mean was accumulated from May 2018 to April 2019. *prwc:* Perennial ryegrass and white clover swards; *prwrc*: Perennial ryegrass, white- and red clover swards; *prwrch*: Perennial ryegrass, white- and red clover + herbs.

Despite no grazing events, some appreciable emissions (up to about 1.0 mg N m^{-2} d^{-1}) also occurred during the winter months. The mean (±se) annual N_2O-N emissions were 0.11 ± 0.01 kg N ha^{-1} and 1.0 ± 0.08 kg N ha^{-1} for the ungrazed and grazed pastures, respectively, with the differences between the pastures being insignificant (Figure 3.3; $p > 0.05$). The 8-month (May–December) average was slightly higher for 2018 compared with 2019 (0.71 ± 0.06 vs 0.56 ± 0.11 kg N ha^{-1}) with the differences between pastures and years or their interaction being insignificant (Figure 3.3; $p > 0.05$).

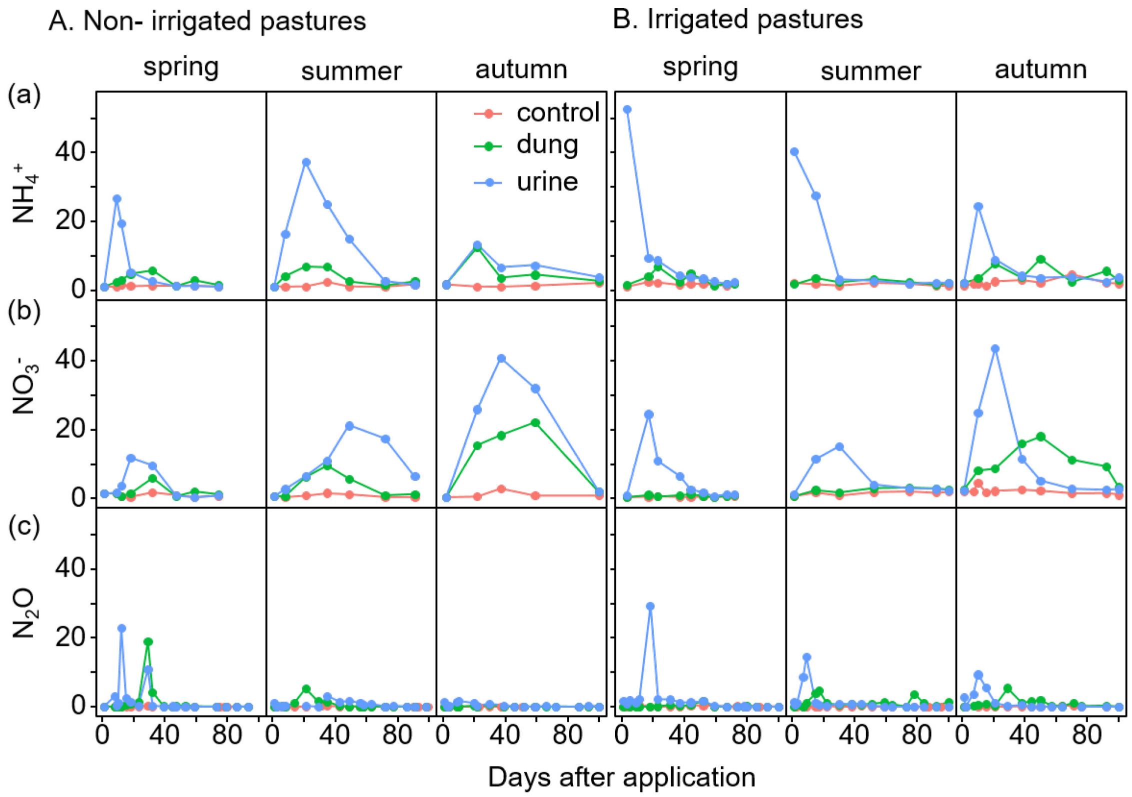

Figure 3.4. Changes in the soil a) ammonium (mg N kg^{-1} dry soil), and (b) nitrate contents (mg N kg^{-1} dry soil) plus daily emissions of (c) nitrous oxide (mg N m^{-2} d^{-1}) from A. non-irrigated pastures pooled across grasslands and B. irrigated pastures.

3.3.2.2 N_2O emission from dung and urine patches (simulated grazing)

3.3.2.2.1 Temporal nitrogen fluxes

Excreta application increased soil mineral N concentration in soils of non-irrigated and irrigated pastures across seasons, being higher for urine than dung (Figure 3.4A, B). Each excreta application resulted within the next days in a large increase in soil NH_4 and subsequently, in soil NO_3, but with varying magnitudes depending on the season and excreta type (Figure 3.4). As expected, the incidence of NH_4 peaks occurred earlier than NO_3 peaks as urine is first transformed into NH_4 and then into NO_3. Therefore, NO_3 flux from spring-applied urine patches was higher for irrigated pastures compared with the non-irrigated ones. For the non-irrigated pastures, summer excreta application resulted in the highest and most extended peaks of NH_4 concentrations when compared with spring and autumn (Figure 3.4A (a)).

NO_3 concentrations returned to background levels within the same timeframe as NH_4 in spring but appeared to persist over a more extended period in summer and autumn (Figure 3.4A(b)). Daily N_2O-N fluxes ranged between −0.2–24.2 mg m^{-2} d^{-1} with the highest peaks in spring from urine patches (Figure 3.4A(c)). The fluxes were back to background levels between 1–3 months, depending on the season (Figure 3.4A(c)). Subsequent fluxes following excreta application in 2018, particularly in summer and autumn, coincided with precipitation events after the initial peak fluxes (Figure 3.1). The highest NH_4 peak from the irrigated pastures occurred in spring but with a shorter lifespan than the other seasons (Figure 3.4B (a)). NO_3 concentrations in summer levelled to the background within the same time frame as NH_4 (Figure 3.4B (a,b)). Whereas NO_3 concentrations in soils were affected by the excreta type and excreta deposition timing, NH_4 concentrations were only affected by the excreta type (Table 3.3). Both NO_3 and NH_4 concentrations were highest, intermediate, and lowest for urine, dung, and control plots. NO_3 concentrations were three-four times lower when excreta was applied in spring or summer than autumn ($p < 0.05$). Daily N_2O-N fluxes ranged from −5–293 g ha^{-1} d^{-1} with the highest peak in spring from urine patches (Figure 3.4B(c)). Generally, peak N_2O-N fluxes' incidences were earlier from urine patches than from dung patches following the excreta application in all seasons. The fluxes were back to background levels between 1–3 months, depending on the season (Figure 3.4B(c)).

3.3.2.2.2 Cumulative N_2O emissions

Annual N_2O-N emission from Experiment 2 (non-irrigated grasslands) following 2018 excreta applications ranged from 0.071–0.130, 0.288–1.69, and 0.515–1.70 g ha^{-1} d^{-1} for control, dung, and urine patches, respectively. All the N_2O emissions were similar across grasslands, but N_2O emissions were affected by the type of excreta or time of application ($p < 0.05$; Table 3.3; Appendix B, Table B2). Accordingly, cumulative N_2O-N emissions were 8–10 times higher for urine or dung treatments than the control and were highest in spring and lowest in autumn, with 420 and 230 g N ha^{-1} fewer emissions in summer and autumn, respectively.

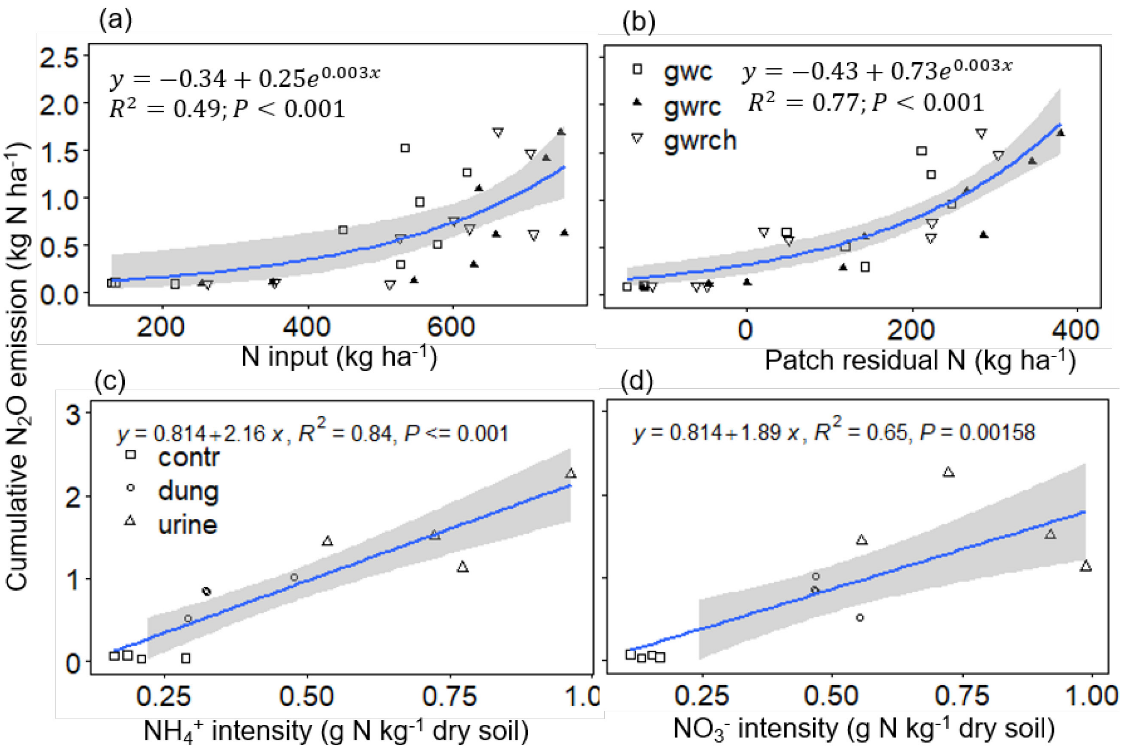

Figure 3.5. Best models showing stoichiometric relationships between cumulative N_2O-N emission and (*a*) N input, (*b*) Patch residual N, (*c*) ammonium intensity of (*d*) nitrate intensity. a-b based on data from experiment 2, *c*-*d* based on the irrigated experiment (3); the grey region is 95% confidence interval.

However, grassland and season or treatment interaction effects were not significant ($p > 0.05$; Appendix B, Table B2). Also, N_2O-N emissions per unit N uptake was significantly affected ($p < 0.05$) by the excreta type and season of excreta application (Table 3.3; Appendix B, Table B2). Accordingly, this parameter was similar for urine and dung but higher than the control and was highest, intermediate, and lowest in spring, summer, and autumn, respectively.

Cumulative N_2O-N emissions from Experiment 3 (irrigated pastures, sampled for 100-days) ranged from 0.02–0.09, 0.256–1.118, and 0.958–2.615 kg ha^{-1} from control, dung, and urine treatments (Table 3.3) with mean ± SD of 0.81 ± 0.91 kg ha^{-1}. The cumulative N_2O-N emissions from non-irrigated similar swards and a similar sampling duration were 0.46 ± 0.55 kg ha^{-1} (mean ± SD). The cumulative N_2O emissions were significantly ($p < 0.01$) affected by the treatment type and treatment-season interaction effects (Appendix B, Table B2). Therefore, whereas cumulative N_2O emissions were higher for urine patches than dung in spring, the emissions were similar in summer and autumn but higher than the control plots (Appendix B, Figure B1). Whereas cumulative urine N_2O emissions were higher in spring than summer or autumn, the dung N_2O emission only differed between spring and summer (Appendix B, Figure B1).

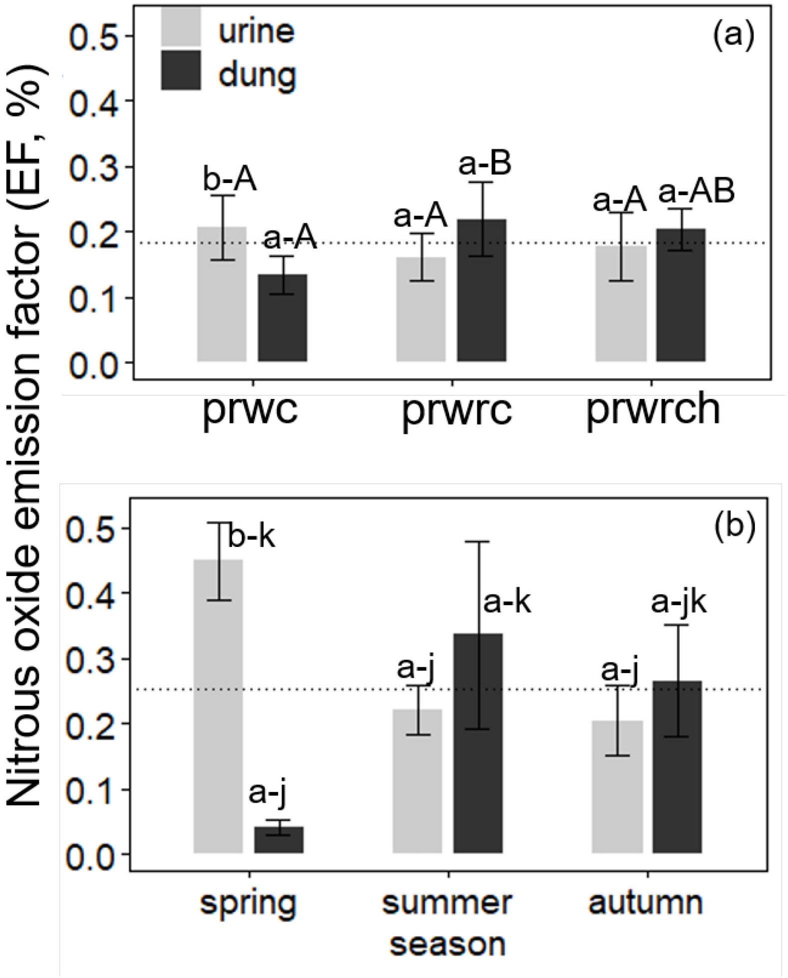

Figure 3.6. Bar charts showing N_2O-N EF (% of applied excreta N emitted as N_2O-N) as affected by (a) grassland x treatment interaction effect in non-irrigated pastures and (b) season x treatment interaction effect in irrigated pastures. Means with different letters are significantly different [$P<0.05$; ^{ABC} being grassland effect, ^{jkl} being seasonal effect and ^{abc} being treatment effect].

3.3.2.2.2.1 Drivers of N_2O emission

We fitted linear and quadratic models around the measured cumulative N_2O emission from excreta patches using the botanical or soil N variables as predictors. For the non-irrigated pastures, an exponential model involving N input as the predictor variable explained 49% of the variations in annual N_2O emission (Figure 3.5a). However, predicting annual N_2O-N emissions using PRN (Figure 3.5b) yielded a relatively higher coefficient of determination ($R^2 = 0.77$). The cumulative N_2O-N emissions from the irrigated pastures were best described by the mineral N intensity across seasons, with a higher coefficient of determination for the NH_4-N intensity than the NO_3-N intensity ($R^2 = 0.84$ vs 0.65; Figure 3.5c,d).

3.3.2.2.3 N_2O emission factors

N_2O-N EFs were calculated based on 100 days of sampling, higher than the IPPC recommended 30 days threshold. Calculating N_2O-N EF based on (Equation (5)) reduced the N_2O-N

EFs by 16% across grasslands, excreta type, and seasons of its application (Table 3.2). N_2O-N EFs for the non-irrigated and irrigated pastures ranged from 0.01–0.35% (mean ± SD = 0.16 ± 0.11%) and 0.04–0.45% (0.25 ± 0.14%), respectively. For the non-irrigated swards, N_2O-N EF was significant for the pasture and N source interaction ($p < 0.05$; Appendix B, Table B2, Figure 3.6a), with *prwc* swards having lower EF for dung compared with *prwrc.* There were treatment and season interaction effects ($p < 0.01$; Appendix B, Table B2) with the irrigated swards. Therefore, whereas the treatment effect was only evident in spring, N_2O-N EF was only higher for dung in summer than in spring (Figure 3.6b).

3.3.2.2.3.1 Drivers of N_2O-N EF

We tested the effect of the measured/estimated parameters on the calculated N_2O-N EF using data from Experiments 2 and 3 (the results are shown in Appendix AB, Table B3). We found that the soil temperature, precipitation, excreta N and C content, N loading, NO_3-N intensity, legume and grass proportions in the swards, sward N uptake, and PRN significantly ($p < 0.05$) affected N_2O EFs for the non-irrigated swards. However, for the irrigated swards, excreta C and N, N applied, and soil mineral N intensity, as well as the dry matter yield, significantly affected N_2O-N EF ($p < 0.05$; Appendix B, Table B3). In addition, the increasing grass proportions resulted in lower N_2O-N EF (Figure 3.7a), while legume proportions >40% resulted in increased N_2O-N EF (Figure 3.7b).

As expected, the N uptake (Figure 3.7c) was negatively correlated with N_2O-N EF, while PRN associated positively with a higher N_2O-N EF ($p < 0.05$; Table 3.3; Figure 3.7d). Whereas soil temperature was significantly and linearly associated with non-irrigated N_2O-N EFs, the environmental factors did not associate significantly with irrigated N_2O-N EFs. However, using pooled data from both experiments for conducted regression approaches showed that WFPS and soil temperature were most related to N_2O-N EF within the first 15 and 60 days following excreta application, respectively, and were quadratic functions (Appendix B, Table B4; Figure 3.7e, f). It appears N_2O-N EF only increased when WFPS increased above 60% (Figure 3.7f) and when soil temperatures increased above 15 °C (Figure 3.7f).

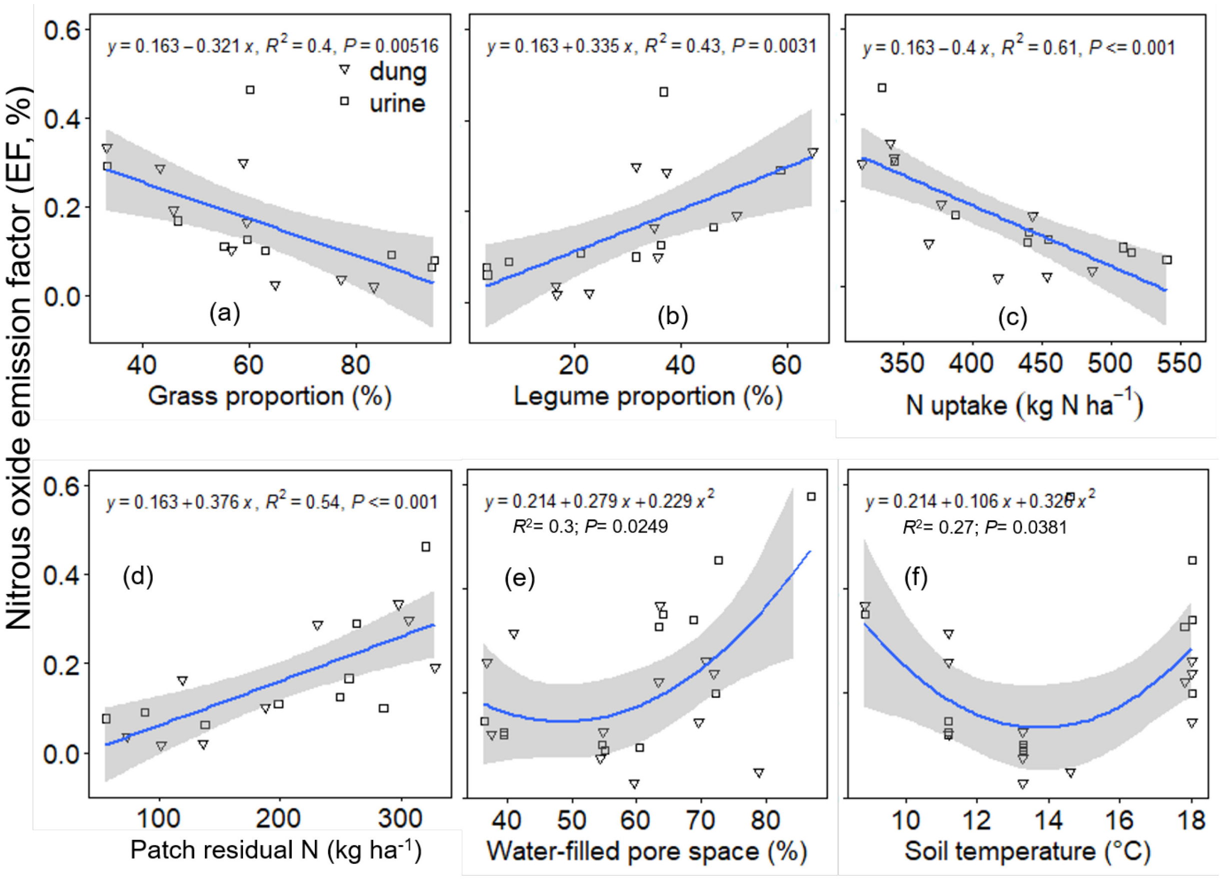

Figure 3.7. Graphs showing stoichiometric relationships between N_2O-N EF from pastures and (a) grass or (b) legume proportion, (c) N uptake, (d) PRN, (e) water-filled pore space and (f) soil temperature. The grey region is 95% confidence interval. *a–d* were pooled across grasslands; *e–f* pooled across experiments 2 and 3. Individual dots indicate replicates of dung and urine patches.

3.4. Discussion

3.4.1 Plant yields, botanical composition and soil residual N

We did not consider yields and forage uptake from the grazed plots (Experiment 1). However, a previous report (LORENZ *et al.*, 2020) showed high forage production from these fields (9.86–11.64 Mg ha^{-1} year^{-1}). As expected, dry matter forage production and N uptake were higher in the diverse *prwrch* pastures than the binary *prwc* pastures in Experiment 2. The higher BFN inputs might partly explain this difference in the *prwrch* pastures due to their higher legume (N) yield (Table 3.3). The similar dry matter production of *prwrch* and *prwrc* despite the higher N input in the mixture without herbs might be due to the higher ability of multispecies swards to utilize resources for growth efficiently. Previous studies reported higher below and above-ground biomass yield more diverse than less diverse swards (CONG *et al.*, 2017). The particularly low dry matter yield of *prwc* pastures can be partly explained by the inability of white clover-containing swards to withstand drought (KOMAINDA *et al*., 2020). The summer of 2018 was especially dry but had an extremely productive spring and an extremely productive long autumn, with excellent growing conditions for white clover. As a result, white clover suffered only six weeks of drought. The range of dry matter yield observed in this study agrees with the 9–12 Mg ha^{-1} reported for similar grasslands under similar climatic and soil conditions, depending on the age or soil N status (LORENZ *et al*., 2020; REINSCH *et al*., 2020).

As the N concentration of both urine and dung could not be controlled, N loading rates varied across seasons and treatments with means (±SD) of 493 ± 43 and 397 ± 47 kg ha^{-1} for urine and dung patches, respectively, but were similar across grasslands. Still, these differences appear negligible compared to the range of N loading rates of 200–2000 kg N ha^{-1} year^{-1} from urine and dung patches reported for dairy cows (SELBIE *et al*., 2015). The excreta application increased the grass proportion in the swards but reduced the legume yield as N became more available (VINTHER, 2020). With this, the partner grass outcompetes the legume easily (LEDGARD & STEELE, 1992), especially the shallow-rooted white clover. This same argument could be offered to explain the low legume yield by *prwc* (Table 3.3).

BFN in grass/legume swards worldwide ranges 13– 682 kg N ha^{-1} year^{-1}, depending on the legume persistence, yield, associated grass(es), or measurement method. Therefore, the BFN range (51–286 kg N ha^{-1} year^{-1}) observed in this study could be considered normal. Furthermore, the N applied and N input correlated positively (but weakly; r = 0.27–0.33), while the dry matter yield, legume proportion, and BFN correlated more strongly (r = 0.63–0.76). This observation confirmed the critical role of BFN in dry matter production and supported the assertion that legumes deliver a more synchronized N to meet plant demand compared with the N supply from external sources and stimulate the over-yielding of biomass (FUCHS *et al*., 2020; LÜSCHER *et al*., 2014). Besides, the relatively high dry matter yield from the control plots compared with dung and urine treatments (Table 3.3) further corroborates this assertion. Indeed, LEDGARD (2001) contended that pastoral systems could rely solely on BFN for moderate to high dry matter production with moderate N losses.

3.4.2 N_2O emissions and emission factors

N_2O emissions from grazed pastures are associated with substantial heterogeneity due to the random nature of emission hotspots from urine and dung depositions. This makes the eddy covariance method preferred to the chamber method (VOGLMEIER *et al*., 2019) for non-simulated grazing experimental designs. However, the chamber method can capture the magnitude and temporal patterns from emission hotspots (i.e., urine and dung patches), making the comparison of different treatments in parallel possible. Moreover, the accuracy of measurements can be improved if the interval of measurements is increased (MCAULIFFE *et al.*, 2020). Nevertheless, the high coefficient of variation (about 23%) associated with the current measurements might have undermined the detection of significant differences. Due to this, an additional experiment with controlled urine and dung depositions was designed, which allowed creating more uniform N loadings and tracking the emissions from exactly one urine or dung patch.

The range of daily N_2O-N fluxes observed from our grazed pastures was similar to the 1.1–2.9 mg N m^{-2} d^{-1} reported for grazed Irish pastures on sandy soils (RAFIQUE *et al.*, 2011) and 0.03–2.5 mg N_2O m^{-2} d^{-1} reported in New Zealand (SAGGAR *et al.*, 2004). However, the range of annual N_2O-N emission observed for the grazed pastures (mean ± SD = 1.0 ± 0.2 kg N ha^{-1}) appears lower than the values reported by these earlier authors due to the infrequent incidence of high peaks (Figure 3.2). In addition, the pastures in these previous studies received additional chemical N fertilizers and were conducted in locations with slightly higher temperatures compared with our site. These factors might explain the lower fluxes in our case since farm management contributes to site characteristics and climate variability on the N_2O emission (REES *et al.*, 2013). Nevertheless, the result agrees with the 0.04–21.21 kg N_2O-N ha^{-1} $year^{-1}$ reported for European grasslands (Jena Experiment), depending on the site and management (REES *et al.*, 2013).

The range of cumulative N_2O-N fluxes (0.05–1.59 kg N ha^{-1}) observed in this study from experiment 2 was slightly lower than the 0.08–3.2 kg N ha^{-1} reported for similar simulated grazing experiments (BOWATTE *et al.*, 2018; LUO *et al.*, 2018), probably since these previous authors used mono-crop swards. The N_2O EFs observed for urine and dung in the current study might be related to the soil, climate, and management practices. Previous studies conducted in similar climates (CHADWICK *et al.*, 2018; KROL *et al.*, 2016; SIMON *et al.*, 2018; THOMAS *et al.*, 2017; VOGLMEIER *et al.*, 2019) reported low EFs for urine and dung in line with our findings. DE KLEIN *et al.* (2014) and MARSDEN *et al.* (2016) reported low (0.03–0.3%) N_2O-N EF for animal excreta deposited on free-draining soils. The soils at the site are free-draining sandy loam (Appendix B, Table B1) which must have increased infiltration and percolation of urine, leading to increased N losses via leaching (SIMON *et al.*, 2018) and reduced N_2O emission. Recently, low N_2O-N EFs (0.12–0.41%), depending on the age of sward and fertilization (slurry) rate, was observed for grass-legume grasslands at the study site (REINSCH *et al.*, 2020). As the current study measured N_2O emissions for at least 100 days, the N_2O-N EFs could be considered site-specific. However, the summer of 2018 was unusually dry, though spring was typical, and autumn very productive.

When N_2O-N EF was estimated based on the total N input (i.e., N_2O-N EF_1 = BFN + N applied), to account for the contribution from legumes in the swards, the mean EF reduced by 16% but was not significantly ($p > 0.05$) affected by the sward diversity nor excreta type (Table 3.3). However, this method of estimating EF contrasts with the IPCC 2006 guidelines, which requires that background emissions are considered. Nevertheless, this method provides an opportunity to illustrate the impact of legumes on N_2O emission and might guide the maximal N input for an appreciable level of N_2O emission from grass-legume swards (REINSCH *et al.*, 2020). The N_2O EFs from our study were relatively low compared with the revised IPCC default EF of 0.77% (IPCC, 2019).

HOLTAN-HARTWIG *et al.* (2002) observed that some soils generally have the intrinsic potential for low N_2O emission irrespective of environmental conditions, and this has been attributed to differences in denitrifying communities (CAVIGELLI & ROBERTSON, 2001). Moreover, previous and current soil management practices might affect microbial dynamics in soils. The initial N status of soils is likely to have reverberating effects on the microbial soil community's functions, including N transformations, abundance, and the activity of nitrifying and denitrifying microorganisms (LEVY-BOOTH *et al.*, 2014), and by extension, the production of N_2O. The microbial community's composition is an essential factor determining the N_2O: N_2 ratio emitted from temperate soils (SAGGAR *et al.*, 2013). The soils were not fertilized and must have had a low residual N, as shown by the control plots' negative PRN (Table 3.3).

3.4.2.1 The effect of grassland diversity

We expected a lower N_2O emission from the most diverse pasture (*prwrch*) under grazing and simulated grazing experiments. This expectation was based on the assumption that diverse pastures increase plant productivity through increased niche complementarity, resource uptake efficiency, and N transfer from legumes to grasses/herbs, and particularly, as these also contained plants reported to reduce N_2O emission via nitrification inhibitors (BOWATTE *et al.*, 2018; LUO *et al.*, 2018). A decreasing trend in N_2O emission as grassland diversity increased was observed from grazed plots in 2019 (Figure 3.3), and when excreta N_2O emitted was related to the N uptake (Table 3.3). However, the annual N_2O, N_2O EF or N_2O emission intensity were statistically similar for the grasslands. Moreover, the timing of peak N_2O production appeared similar across grasslands, seasons, and treatments. This observation contradicts reports by HOEFT *et al.* (2012), who observed alterations in the timing of peak N_2O production after urine addition due to species differences. Although the N input differed among the grasslands, obviously due to the differences in legume proportions, N_2O emission was similar across the grasslands. In addition, the mineral N concentration was similar for the grassland soils (Table 3.3), suggesting that grasslands might not be too different functionally to influence N cycling. Therefore, we reject the hypothesis that a more diverse grassland would necessarily reduce the N_2O emission.

The effect of sward diversity on N_2O emission is not conclusive. (HOEFT *et al.* (2012) reported no difference in the N_2O emission between grass pastures and more diverse pastures in previous studies. In contrast, NIKLAUS *et al.* (2016) observed reduced N_2O emission from more diverse pastures (species richness from 1–16) but not in the presence of fertilizer or large proportions of legumes. In this study, herbs constituted about 18% of the most diverse swards and forage plantain proportion was only around 5% (results not shown). This herb proportion is likely too low for any effects on N_2O emissions. Besides, the soil-environment interaction might affect plantain BNI effectiveness (LUO *et al.*, 2018; PODOLYAN *et al.*, 2020). According to *Experiment 2*, the binary pasture yielded less dry matter and less nitrogen (Table 3.3) and had possibly a higher uptake by the animals, which would have resulted in higher droppings of urine and dung. Therefore, similar N_2O emissions from higher N-yields in the more complex grasslands could be considered positive.

3.4.2.2. Effect of N source

The effect of excreta type on the N_2O emission was not consistent as the interaction effects were evident for both non-irrigated and irrigated pastures. However, PRN and all the N_2O emission indices were generally similar for both dung and urine in non-irrigated pastures, but with irrigation, N_2O emissions/EFs were 1–2 times higher for urine patches compared with dung. LUO *et al.* (2018) also reported a higher N_2O emission from urine patches relative to dung, and this could be ascribed to the slow mineralization of dung N. Whereas urine contains high amounts of available N, dung is rich in C and organic N, and undergoes slower mineralization rates and higher fixation of N by adsorption to soil particles, depending on soil properties and environmental conditions (VAN GROENIGEN *et al.*, 2005). However, the N mineralization from dung patches and subsequent emission of N_2O might be a function of biological, environmental conditions, and time, as over time immobilized N becomes available for mineralization resulting in a later peak, as occurred in experiment 2. It is not clear if all N from dung patches mineralized during the period as dung N were bound in very slowly decaying organic bindings, or more complex molecules might need more time than urine, in which most N is in mineral form. The relatively dry soil conditions in 2018 might have limited N_2O emission from the urine patches. The drought must have slowed down mineralization even further in dung patches as they remained visibly dry in the collars.

3.4.2.3 Effect of timing of excreta deposition

The peak N_2O emissions were visually associated with a decline in NH_4 and increased NO_3 concentration in the soils. The highest N_2O-N peak was obtained in spring, followed by summer, and then autumn in both years, similar to previous reports (KROL *et al.*, 2016; LUO *et al.*, 2018; VAN DER WEERDEN *et al.*, 2011). The seasonal effect on PRN, N_2O-N emission, and N_2O-N EF might be due to the prevailing soil moisture and temperature interaction effects at or following excreta deposition (VAN DER WEERDEN *et al.*, 2011). These environmental differences influence the N uptake and residual N in the soils, and hence N-leaching to the groundwater, which is a function of timing of excreta deposition, soil inorganic N status, and climatic conditions (BIERNAT *et al.*, 2020). Although the leaching load was not measured in this study, BIERNAT *et al.* (2020) reported higher leaching loads when soil mineral N was high in late summer, autumn or early winter. This observation might partly explain the low residual N or N_2O emission associated with summer and autumn excreta applications relative to spring.

3.4.2.3 Effect of irrigation

The irrigated pastures in Experiment 3 had relatively high WFPS compared with Experiment 2 (Figure 3.1). It is worth mentioning that irrigation was adopted to provide conducive soil conditions for denitrification losses. Such high soil moisture conditions could promote plant growth and enhance plant N uptake and promote N leaching to the underground water pool. Similar to reports by SMIT *et al.* (2020), the irrigation increased (almost doubled) both cumulative N_2O-N emitted (0.46 vs 0.81) and N_2O-N EF (0.25 vs 0.16%) compared with non-irrigation emissions. The dry conditions in 2018 might have reduced N mineralization, particularly in dung patches, as they were visibly dry and would have limited denitrification. With the irrigated swards, both nitrification and denitrification were significant processes by which N_2O fluxes were produced; nitrification seemed to be more dominant, as shown by the more robust relationship between N_2O and NH_4 compared with NO_3 ($R^2 = 0.84$ vs 0.65; Figure 3.5c,d; SIMON *et al.*, 2018).

3.4.3. Factors that controlled N_2O emission and EF at the site

3.4.3.1 Botanical characteristics

The measured/estimated botanical variables were significantly associated with N_2O-N EFs in this study. The impact of plants on N_2O emissions and underlying mechanisms are not always conclusive (DE KLEIN *et al.*, 2019), as several factors might play confounding roles. However, grasslands' ability to influence N_2O emission might depend on factors including N uptake from soils, legume proportion, and the production of soluble sugars (DE KLEIN *et al.*, 2019; NIKLAUS *et al.*, 2016). Regression analyses showed a significant effect of grass proportion, legume proportion, and N uptake on N_2O-N EFs (Appendix B, Table B3, Figure 3.7a–c). The higher grass proportion and N uptake appeared to depress the N_2O emission factor, but the higher legume proportion associated positively with N_2O-N EF. Accordingly, BRACKEN *et al.* (2020) reported a significant functional group identity effect on cumulative N_2O emissions, with emissions increasing with the increasing white clover proportion and decreasing with the increasing perennial ryegrass proportion for both irrigated and non-irrigated pastures. Similarly, NIKLAUS *et al.* (2016) reported a significant legume effect on N_2O emission. On the contrary, BARNEZE *et al.* (2020) found no legume effect on N_2O emission, total mineral N or mineral N in fertilized or unfertilized soils.

Under monoculture, legumes appeared to emit more N_2O than other species (NIKLAUS *et al.*, 2016). However, this might not be necessarily additive in mixed pastures (ABALOS *et al.*, 2014), but increases in the proportion of legumes might influence C and N cycling processes (BARNEZE *et al.*, 2020). This legume effect on N_2O emission might be related to the increased N supply (Table 3.3), leading to nitrification and denitrification losses. Therefore, the increased NO_3 availability due to the release of fixed N from legume roots via decomposition and legumes' inefficiency in acquiring mineral N (BARNEZE *et al.*, 2020) may explain the high association of legumes with N_2O emission. The inclusion of legumes in mixed-swards at proportions of 30–50% is described as most effective, providing multiple outputs including reduced greenhouse gas emission, higher productivity and increased protein self-sufficiency, and reduced N leaching (LÜSCHER *et al.*, 2014; VOGELER *et al.*, 2019). In this study, legume proportions ranging from 30–50% resulted in N_2O-N EFs ranging from 0.17–0.25% (Figure

3.7b). Meanwhile, legume proportions of up to 30% have been widely promoted in New Zealand and Switzerland as a norm (FUCHS *et al.*, 2020).

3.4.3.2 Soil mineral N availability and plant N uptake

In our study, soil mineral N intensity, PRN, and N uptake were significantly associated with N_2O-N EF. The N input was 116–289 kg ha^{-1}, more than the 300 kg N ha^{-1} required by highly productive grasslands to meet plant demand (RAFIQUE *et al.*, 2011). At the same time, the N uptake (320–540 kg ha^{-1}) was lower than the N input, resulting in patch residual N ranging from −87–206 (mean = 103 kg ha^{-1}), being positive for excreta patches (Table 3.3) and negative for the control plots. This estimate might not be precise as lateral flows from urine and dung patches outside the rings were possible (CICHOTA *et al.*, 2018). Also, N losses via nitrification, denitrification, and leaching were not considered. Moreover, these pastures can sequester about 1 Mg C per year, equivalent to ~100 kg N (assuming a CN ratio of 10), captured in soil organic matter. Nonetheless, the residual soil N on the entire pasture must be far lower, considering that excreta deposition by grazing livestock affects about 60% of the paddock.

In any case, N_2O production is primarily driven by the amount of residual N in the soil (SAGGAR *et al.*, 2013), as corroborated by the high association between N_2O emission and PRN ($R^2 = 0.77$; $p < 0.001$; Figure 3.5b), which is a function of N input and uptake of the swards. The higher residual N will potentially result in higher N_2O emission as N becomes available for nitrifies and denitrifies to utilize. It appears values up to 250 kg ha^{-1} might still keep N_2O-N EF from cow excreta patches below 0.2% (Figure 3.5b) at this site, but the high surplus N might be leached to pollute the underground water pool and/or become a secondary source of N_2O emission. PRN was about 67% higher for *prwrc* than *prwc* or *prwrc*, leading to a relatively low N uptake for *prwrc.* The similar N uptake for *prwrc* and *prwrch* might be due to the high grass proportion (Table 3.3) as grasses have a higher potential to utilize soil N for growth, reducing soil N and the nitrification/denitrification potential of the soils *(*BOWATTE *et al.*, 2018). The range of N uptake by the grasslands in this study appears to be on the upper side of the range of values (10–450 kg N ha^{-1} year^{-1}) reported by BESSLER *et al.*, 2012). It appears grass-legume swards are associated with high N uptake, as corroborated in this study and by REINSCH *et al.* (2020). This high N uptake potential has been attributed to species complementarity, evident by the development of high root length and root density by grass-legume species (CHEN *et al.*, 2016), leading to low PRN.

The high N uptake (>70%) in this study might partly explain the low N_2O-N EFs observed in this study, as the higher N uptake was significantly associated with low N_2O-N EF (Figure 3.7c). The higher N uptake reduced N_2O EF, and the higher N input might stimulate the higher N uptake, as observed in this study (results not shown; $r = 0.44$, $p < 0.05$), similar to reports by LÜ *et al*. (2014). This observation supports the general concept that the nutrient uptake by roots increases in response to increasing nutrient supply (LAMBERS *et al*., 2008). Besides, this highlights the critical role of plant N uptake and its potential usage in mediating the effects of N addition from grazing animals. However, it must be noted that plant N uptake/productivity also depends on other nutrients and has a physiological maximum. Although N_2O emissions were similar across the grasslands, the most diverse grassland had a relatively high N uptake compared with the binary-sward grassland, suggesting a higher potential for reduced N_2O emission as corroborated by the 9% lower N_2O emission intensity by *prwrch* compared with *prwc* (Table 3.3).

3.4.3.3 Soil moisture and temperature

As shown by regression analyses, soil moisture and temperature were significant predictors of N_2O-N EF for both non-irrigated and irrigated pastures but appeared to be most vital within the first two weeks and two months, respectively, following excreta application (Appendix B, Table B4). Soil moisture and ambient temperature influence N_2O emission significantly by modulating the microbial activity and transport of gases in soil (MCAULIFFE *et al.*, 2020; VAN DER WEERDEN *et al*., 2014). Higher N_2O-N EFs associated positively with higher WFPS. However, it appears that increases in N_2O-N EF only occurred when WFPS was above 60%, respectively (Figure 3.7e). Soil moisture affects nitrification and denitrification processes (VAN DER WEERDEN *et al*., 2014), and for that matter, ammonia-oxidizing species (DI *et al*., 2014) reduce their population below 60% of field capacity. The soil water threshold value differs according to the soil type (SAGGAR *et al*., 2013), and the critical WFPS for denitrification in many soils is equivalent to the field capacity or above (DE KLEIN & VAN LOGTESTIJN, 1996). At higher WFPS, where denitrification becomes the primary process, the N_2 produced might exceed the N_2O production, reducing N_2O emissions at high WFPS, especially when temperatures are high. (DAVIDSON, 1991) explained that both nitrification and denitrification processes co-occur within soils, but the low soil water content and coarse soil texture favour nitrification. In contrast, the high soil water content and fine soil texture with a high organic matter content promote denitrification (DE KLEIN & ECKARD, 2008). The seemingly high occurrence of nitrification might be due to the sandy nature of soils at the site, partly explaining the relatively low N_2O emission observed in this study.

Higher N_2O-N EF associated positively with higher soil temperature, but it appears increases in N_2O-N EF only occurred when the soil temperatures went above 15 °C (Figure 3.7f). MAAG & VINTHER (1996) reported maximum nitrification rates at 20 °C and small responses to soil temperature changes at lower temperatures. Although denitrification depends on the soil type, with sandy loam soils responding significantly to both increases in soil temperature and soil moisture, temperatures above 5°C are often considered to be required for effective denitrification rates (GRANLI & BØCKMAN, 1995). However, we observed an appreciable N_2O emission from grazed fields in winter and early spring (Figure 3.2), similar to earlier reports (FLESSA et al., 1995). Therefore, the observed N_2O fluxes in winter, despite the absence of grazing activity, might be due to substrates accumulations from the preceding grazing season. Such winter emissions have been attributed to specific freeze/thaw phenomena (BURTON & BEAUCHAMP, 1994). However, HOLTAN-HARTWIG et al. (2002) explained that decreasing temperatures increase the N_2O/N_2 product ratio under anaerobic soil conditions due to differences in the temperature response of N_2O diffusion versus biological process rates.

3.5. Conclusions and implications of the study

In this study, the grasslands' species richness did not significantly affect the N_2O emission, although there was a tendency for low N_2O emission from the multispecies swards when the N_2O emission was expressed fraction of the N uptake. Differences in the swards' functional composition (i.e., grass and forage legumes) were significantly related to the N_2O emission. We observed a high N uptake efficiency by the grasslands, partly explaining the low N_2O emission and EFs at the site. In addition, soil temperature and WFPS, botanical factors, including legume or grass proportions in the swards, and N uptake were dominant factors that drove the N_2O emission from dung and urine patches. The high N uptakes observed for grasslands show their high potential to optimize N use. An elevated WFPS almost doubled the N_2O-N EFs; however, the EF values were below the default IPCC (2019) for wet temperate climates. This observation suggests a further disaggregation of the default N_2O EF value to cater for organic dairy systems in wet temperate regions.

The excreta CN ratio, N applied, total N input, PRN, grass and legume proportions, as well as N uptake, were factors that drove the N_2O emission from dung and urine patches. The high N uptake (>70%), legume proportions (16–46%), as well as soil type might explain the relatively low N_2O EFs observed in this study. Results suggest that simple regression approaches using grass or legume proportions and N uptake can be used to predict the N_2O emission more precisely over a wide range of environments. We further concluded that an efficient proportion of legumes without additional mineral fertilizer use accelerates an efficient N-cycling and is more important than a complex species diversity to reduce undesired N_2O emissions from pastures. However, the year 2018 was unusually dry, and results might be or were probably affected by this. Hence, the experiments or at least the simulated grazing studies should be continued to see the difference to “normal” years.

CHAPTER 4

Nitrous oxide emission from forage plantain and perennial ryegrass swards is affected by resource allocation dynamics belowground

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This chapter has been submitted as an original scientific article to the Plant and Soil Journal. It reports the outcome of a greenhouse experiment that tested the efficacy of forage plantain in reducing nitrous oxide emission and explored the impact of belowground resource allocation on N_2O emission.

Abstract

Plantago lancelota has been hypothesized to reduce nitrous oxide (N_2O) emissions from perennial grasslands. However, soil-plant interactions affecting N_2O production in soils are not well understood, and experimental data are scarce. A greenhouse experiment was conducted in a 3x3 factorial, fully randomized design, comprising three mineral N fertilizer rates (0, 150 and 300 kg N ha^{-1}) applied to monocultures and a binary mixture of *P. lanceolata* and *Lolium perenne*. Measured Parameters included daily N_2O emissions, aboveground (AG) and belowground biomass (BG), N- and C- yields, as well as leucine aminopeptidase (LAP) activity in the soil as an indicator for soil microbial activity. Accumulated N_2O emissions were about two times higher for *P. lanceolata* than for *L. perenne* monocultures or mixtures ($p<0.05$). Monocultures of *P. lanceolata* also obtained lower dry matter (AG+BG) and N yields than *L. perenne* and mixtures ($p<0.001$). As a result, the binary mixtures showed the highest N use efficiency and increased LAP activity. Thus, c allocation dynamics belowground appeared as a potential means by which plants might impact N_2O emission. *Plantago lanceolata* was generally ineffective at reducing N_2O emissions. Resource allocation dynamics belowground appear to have a higher dominance on N_2O emission from soils rather than forage plantain itself.

Keywords: Fertilization; nitrous oxide; perennial ryegrass; forage plantain, nitrification inhibition; northern Germany

4.1 Introduction

Nitrous oxide (N_2O) emissions from agriculture contribute about 60% of global anthropogenic N_2O emissions (SYAKILA & KROEZE, 2011). It has a high global warming (the CO_2 equivalent is 265–298) and stratospheric ozone depletion potential (IPCC, 2013; OENEMA *et al.*, 2009; PORTMANN *et al.*, 2012). Although grasslands provide a range of ecosystem services, such as carbon (C) sequestration, soil erosion prevention, landscape, and biodiversity protection (Taube et al., 2014), they can also be a significant source of N_2O emissions, especially when utilized for ruminant livestock production (VAN DER WEERDEN *et al.*, 2020). Moreover, N_2O emissions from intensively managed grasslands are associated with the highest uncertainty of agricultural greenhouse gas (GHG) emissions (FLECHARD *et al.*, 2007; VAN DER WEERDEN *et al.*, 2020). This high uncertainty results because N_2O emission depends on heterogeneous factors, including climatic factors, soil properties, the soil microbiome and botanical factors (FIRESTONE & DAVIDSON, 1989; NYAMEASEM *et al.*, 2021; VOGLMEIER *et al.*, 2019), complicating the development of mitigation strategies. Thus, a better understanding of the factors resulting in N_2O emissions is required to reduce these emissions effectively.

The most commonly sown grassland species in Europe is perennial ryegrass (*Lolium perenne*) (ABALOS *et al.*, 2018; FRANKOW-LINDBERG *et al.*, 2009) due to its persistence under frequent harvesting and production of high-quality forage; combining it with other species can enhance the productivity of both grassland and animals further while also reducing environmental impacts (ABALOS *et al.*, 2018). Mixed pastures have been reported to have a higher tendency to mitigate N_2O emission due to faster growth and rapid resource acquisition (ABALOS *et al.*, 2014; KRAMER-WALTER *et al.*, 2016; NYAMEASEM *et al.*, 2021). The optimization of agroecosystems to retain more N under intensive management has generally focused on aboveground traits with little focus on belowground plant-soil interactions (ABALOS *et al.*, 2018; DEYN, 2017; VERHEIJEN *et al.*, 2016). Even though roots play critical roles in ecosystems processes, including nutrient cycling and generating microbial niches for N_2O production (BARDGETT *et al.*, 2014), such roles can be more relevant than leaf traits in explaining N_2O producing processes (LEGAY *et al.*, 2014). Some botanical factors that affect the N_2O mitigation potential of pasture may include belowground species diversity (ABALOS *et al.*, 2014), the presence or absence of species capable of nitrification inhibition (NI) (BARDON *et al.*, 2014; COSKUN *et al.*, 2017), rhizosphere priming effect (HAMILTON & FRANK, 2001) and whether the species are conservative or acquisitive (ABALOS *et al.*, 2018). The nitrification inhibiting species in mixed swards are particularly appealing and have received attention, as they can concomitantly provide high-quality forage and reduce environmental effects. One of the most promising species in previous studies has been ribwort plantain (*Plantago lanceolata*), which has exhibited the potential to reduce N_2O emissions in grazed pastures (BRACKEN *et al.*, 2020; GARDINER *et al.*, 2018; JUDSON *et al.*, 2019; SIMON *et al.*, 2019). The results have not been consistent, however, probably due to the large number of factors that affect N_2O emissions, with examples ranging from climatic, edaphic and botanical factors such as the percentage of a species present (LUO *et al.*, 2019; NYAMEASEM *et al.*, 2021; PODOLYAN *et al.*, 2020). Accordingly, ABALOS *et al.* (2018) conducted a study and identified that high biomass production and fast N uptake are the main pathways by which plants impact N_2O emission and cited specific leaf area and root length density as particularly relevant determinants. However, their study was based on grasses only, and hence did not include any species with known NI potential.

Differences in resource allocation belowground by pastures can impact soil microbial activity with important implications for nutrient cycling and ecosystem functioning (HARGREAVES & HOFMOCKEL, 2014). Additionally, N deposition has implications for soil microbial dynamics, as N is essential for their metabolic functioning. When applied N exceeds plant demand, it becomes a substrate for microbial metabolic pathways that can produce (or consume) N_2O (BUTTERBACH-BAHL *et al.*, 2013; LUO *et al.*, 2017). Microorganisms are, therefore, sensitive to changes in their environment and may express enzymes in response to soil N availability. These extracellular enzymes, such as leucine aminopeptidase (LAP), are produced to catalyze chemical reactions that facilitate soil organic matter degradation in order to acquire the necessary resources for microbial metabolic functions (CENINI *et al.*, 2016). The expression of such N acquiring (or N_2O-consuming) enzymes varies according to N concentrations in the soil (CENINI *et al.*, 2016). While the factors that affect microbes' role in N_2O emission are numerous and complex, their population dynamics and expression of enzymes producing and consuming N_2O (SPIRO, 2012) in soils could be essential biomarkers for N_2O emission. A high microbial population in the soil might lead to increased consumption or immobilization of surplus N, especially when N-acquiring microbes are involved, leading to reduced N_2O

emission (DE KLEIN *et al.,* 2019). However, the mechanisms by which plants reduce N_2O emissions are not well understood (DE KLEIN *et al*., 2019).

Consequently, understanding the impact of forage plants on plant functional traits is vital to improving estimates of N_2O emissions from pastures (DE KLEIN *et al*., 2019). Thus, we designed a greenhouse pot experiment with constant soil temperature and moisture and three different mineral fertilizer rates to evaluate the effects of monocultures and mixtures of *L. perenne* and *P. lanceolata* on N_2O emission and aboveground (AG) and belowground (BG) C and N partitioning. Using this setup, we tested the following hypotheses: (i) N_2O emission from *P. lanceolata* swards are lower than in *L. perenne* monocultures due to their expected nitrification inhibiting effect, (ii) N-uptake efficiency plays an essential role in estimating N_2O emissions from pasture soils and (iii) thus, LAP expression is highly associated with N_2O emission, and therefore a suitable biomarker for N_2O emission from pastures.

4.2. Materials and methods

4.2.1 Experimental design

The experiment was conducted in a greenhouse located in the University of Kiel, northern Germany (54°20'N, 10°6'E). The soil was unsterilized topsoil obtained from grazed grass-clover swards obtained from an organic experimental dairy farm. The soil texture was a sandy loam and contained 1.6 % C, 0.13 % N, 8 mg/100 mg P_2SO_5, 10 mg/100 mg K_2O and 6.9 mg/100 mg Mg. The soil's pH was 5.9, and the bulk density was uniformly set to 1.56 g/cm^3. The soil was sieved through a 3 mm mesh, homogenized and placed in circular-shaped pots, measuring 0.14 m and 0.18 m in diameter and depth, respectively. This comparably small size allowed for a better root/soil ratio while being large enough to provide adequate rooting volume for the duration of the experiment, even for the deep-rooting *P. lanceolata*. The botanical composition was varied and included monocultures of *L. perenne* and *P. lanceolata* and their binary mixture.

Plantago lanceolata seeds were pregerminated in Petri dishes, and one viable plant was transplanted into each pot with a *P. lanceolata* treatment to ensure an even distribution of *P. lanceolata* in all pots. As a result, the sowing density of *L. perenne* was the equivalent of 32 kg ha^{-1} for all *L. perenne* containing pots. Before starting the gas measurements, all plants were allowed to establish for approximately one month to ensure viable swards. Subsequently, a standardization cut was performed, which was not measured for dry matter (DM) yields. The experimental units consisted of a 3x3 factorial design, comprising three N fertilizer (urea) rates (0, 150 and 300 kg N ha^{-1} equivalents, subsequently called N0, N150 and N300) applied in three splits (see Figure 4.1), and the three swards types (*L. perenne* (LP), *L. perenne – P. lanceolata* (LP+PL), and *P. lanceolata* (PL)), replicated four times and arranged in a completely randomized block design. The fertilizer was dissolved in 30 mL water and applied by sprinkling from above. The greenhouse was illuminated for 16 hours each day, with daytime temperatures set to 20°C, and nighttime temperatures to 15°C. Volumetric soil moisture was maintained between 0.290–0.330 via capillary irrigation (Ortmann, Votloh, Germany) to ensure optimal growing conditions and was monitored continuously using 5TM volumetric water content sensors coupled with EM 60 loggers (Meter Group, Pullman, WA, USA).

4.2.2 N_2O sampling and measurement

After successfully establishing the swards and the standardization cut, gas sampling started on the 5th of March 2020 and ended on the 27th of May 2020 using static chambers (height = 0.19 m). With the chamber collar sealed using a waterlogged basement, one air sample was taken with a 30-mL polypropylene syringe from the chamber-top at 0, 20, 40, and 60 min after closure. The gas samples were transferred to 12 mL pre-evacuated glass vials (Labco, High Wycombe, UK) and stored for a maximum of one week before analyses. The gas samples were analyzed for N_2O through a gas chromatograph (SCION 456-GC, Bruker, Leiderdorp, Netherlands) equipped with a ^{63}Ni electron-capture detector. Helium was used as the carrier gas and argon-methane as the make-up gas. Three certified N_2O gas standards (300, 620, 1510 ppb) were measured regularly to determine the analytical precision (standard deviation of 10 repeated measurements <3 ppb). The gas chromatograph procedure used a detector temperature of 320 °C, a column temperature of 40 °C and an injector temperature of 200 °C. The samples were injected using an auto-sampler (model 271 LH, Gilson Inc., Middleton, WI, USA). N_2O-N fluxes were calculated by linear regression between measured N_2O-N concentrations and sampling time (PARKIN & VENTEREA, 2010). Accumulated N_2O emissions over the experimental period (83 days) for each treatment and replicate were calculated by linear interpolation between the consecutive measurements.

4.2.3 Biomass sampling and chemical analyses

Aboveground biomass (AG) was harvested from each pot when swards attained a height between 10-12 cm, while *P. lanceolata* achieved flowering stage. The plants were harvested by cutting AG from each pot at 5 cm sward height. At the end of the pot experiment, the sward stubble was removed from the soil surface. The belowground biomass (BG) was separated from the soil with a hydropneumatic elutriation system (Smucker et al., 1982) using a 0.63 mm sieve to separate the roots from soil particles. Floating fine roots were collected with tweezers. All the plant samples (AG, stubble and BG) were oven-dried to a constant weight at 58 °C for 48 hours to determine DM yield. Each dried sample was milled through a 2-mm sieve (Cyclotech mill, Foss analytical, Hilleröd, Denmark), and then N and C contents were measured using a C/N analyzer (Vario Max CN, Elementar, Hanau, Germany). The ratio of N uptake by plants to fertilizer N input constituted N uptake efficiency (NUpE). Shoot N use efficiency was calculated as SNUE = shoot dry weight/shoot N content, and root N use efficiency as RNUE = root dry weight/root N content (JIANG *et al.*, 2016).

4.2.4 Soil sampling and measurement of LAP activity

Soil samples were obtained at each biomass harvest using procedures described by PIJLMAN *et al.* (2020) and ROSEAUX *et al.* (2020). Three soil cores (0–10 cm depth, 1 cm diameter) were randomly sampled from each pot and mixed to obtain a composite sample. A subsample of soil (~2 g) was stored immediately on dry ice and later transferred to a −20°C freezer for microbial enzyme analysis. The resulting hole was filled with a 10 mm diameter PVC pipe and closed off on top to prevent air and light interactions. The kinetics of hydrolytic enzymes involved in the N cycle was measured by fluorimetric microplate assays of 7-amino-4-methyl-coumarin (AMC) (MARX *et al.*, 2005). The leucine aminopeptidase and tyrosine aminopeptidase activities were measured using L-leucine-7-amino-4-methyl coumarin (AMC-L) and L-tyrosine-7-amido-4-methyl-coumarin (AMC-T). All substrates and chemicals were purchased from Merck (Darmstadt, Germany). We determined enzyme activities in a range of substrate concentrations from low to high (0, 10, 20, 30, 40, 50, 100, 200 µmol g^{-1} soil). Saturation concentrations of fluorogenic substrates were determined in preliminary experiments. Suspensions of 0.5 g soil (dry weight equivalent) with 50 mL water were prepared using low-energy sonication (40 J s^{-1} output energy) for 2 min (Stemmer et al., 1998). Then, 50 µL of soil suspension was added to 100 µL substrate solution and 50 µL of buffer [TRIZMA (pH:7.2) buffer for AMC substrate] in a 96-well microplate (KOCH *et al.*, 2007). Fluorescence was measured in microplates at an excitation wavelength of 355 nm and an emission wavelength of 460 nm, slit width of 25 nm, with a Victor 3 1420-050 Multi-Label Counter (Perkin Elmer, USA).

All enzymes were determined and incubated at the exact temperature within 2 hours. After each fluorescence measurement (i.e. after 30 *min*, one *h* and two *h*), the microplates were promptly returned to the climate chambers so that the measurement time did not exceed 2–2.5 min. The assay of each enzyme at each substrate concentration was replicated three times in each plate. Also, for all four incubation replicates, each enzyme's assay at each substrate concentration was performed in three analytical replicates (12 wells in the microplate). The Michaelis-Menten equation was used to determine parameters of the enzyme activity *(V)*:

$$V = \frac{V_{max}\,[S]}{K_m + [S]} \tag{1}$$

where V_{max} is the maximum enzyme activity; K_m represents the half-saturation constant or the substrate concentration at which the reaction rate equals $V_{max}/2$; and S is the substrate amount (MICHAELIS & MENTEN, 1913). Both V_{max} and K_m parameters were approximated by the Michaelis-Menten equation (1) with the non-linear regression routine of STATISTICA.

4.2.5 Statistics

All statistical analyses and graph works were performed using R software (R DEVELOPMENT CORE TEAM, 2019). The measured variables were analyzed using a linear model with pasture type, N fertilization rate, and interaction term as fixed factors. In each case, the adequacy of the model was assessed by examining the appropriateness of residual plots. Effective means were separated at $p<0.05$ with Tukey's post hoc tests, using the "lsmeans" function of the "multcomp" package (BRETZ *et al.*, 2016). Pearson correlation and linear regression tests were performed to establish the relationships among the measured variables. Correlation coefficients between variables, and their significance, were computed using the "sjPlot" package by pairwise deletion procedure. The Lavaan package in R (ROSSEEL, 2012) was used to perform structural equation modelling (SEM) to assess the measured plant traits' direct and indirect controls on N_2O emissions. A conceptual model based on the hypotheses and theoretical knowledge of plant effects on N_2O emissions was constructed. Before the SEM analyses, the predictor and dependent parameters' units were adjusted to obtain comparable parameter variances. Non-significant relationships were removed following a stepwise procedure using model modification indices, (DE VRIES & BARDGETT, 2016). The quality of the SEM model (GRACE, 2006) was determined using the chi-square goodness-of-fit statistic ($P > .05$, which indicated statistically significant model fit), the root means square error of approximation value (RMSEA, <0.08), the comparative fit index (CFI, >0.95), the standardized root mean square residual (SRMR, <0.08).

4.3 Results

4.3.1 Biomass allocation and CN partitioning

Nitrogen application increased the total biomass yield of AG + BG approximately 4-fold (Table 4.1) when compared with the control treatment ($p<0.05$). However, there were no significant differences in the total yields between the N150 and N300 treatments. Only at the third harvest did *P. lanceolata* DM yield increase with N rate, where it was 2-fold higher for N300.

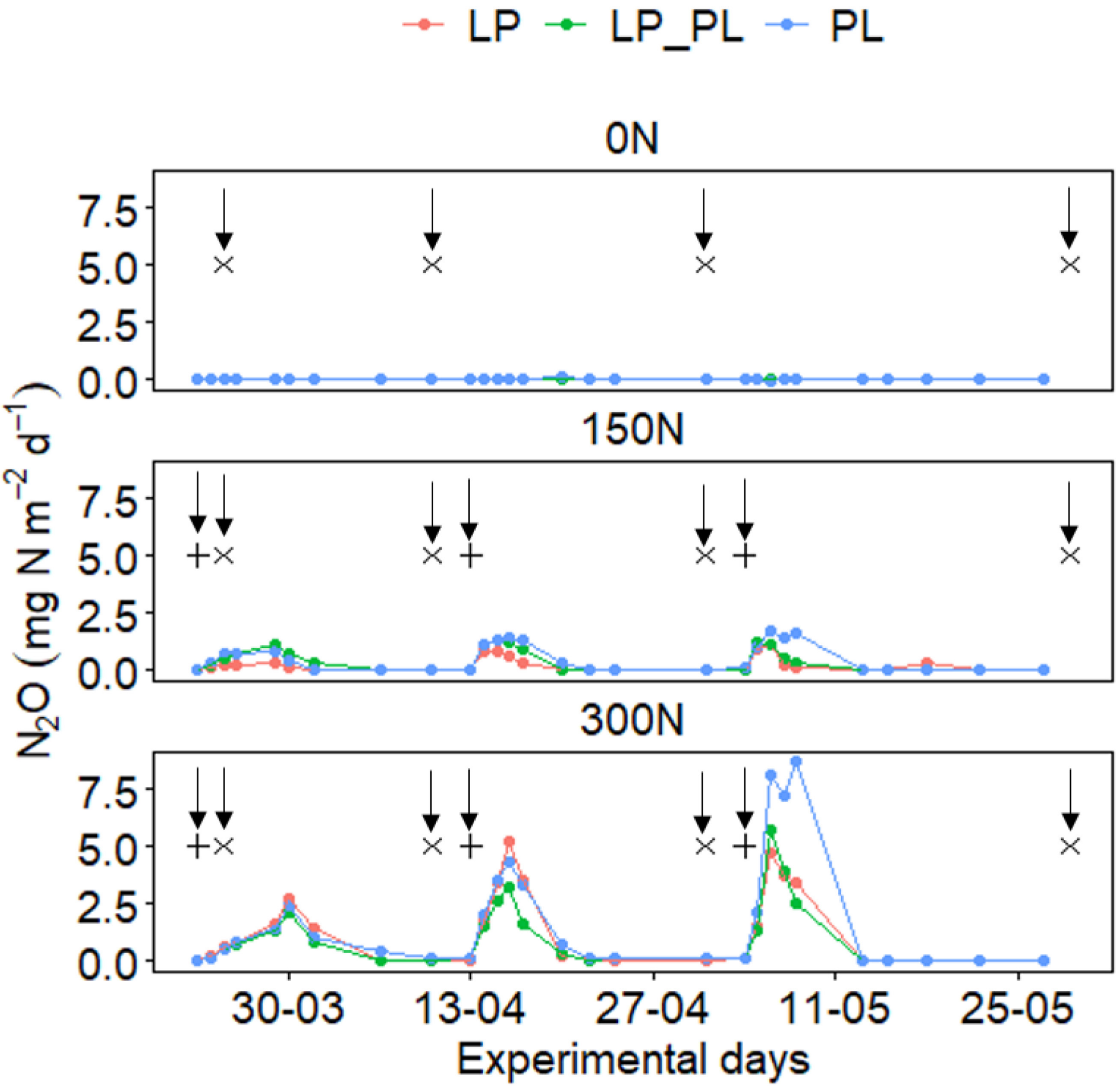


Figure 4.1: Graphs showing daily N_2O fluxes from the diverse swards at different fertilization rates. LP represents *Lolium perenne*; PL represents *Plantago lanceolate*. [Arrows with + represent fertilizer application event and arrows with x represent biomass harvesting event]

Table 4.1: Dry matter yield, C: N ratios and N uptake/use efficiencies from *Lolium perenne* (LP) and *Plantago lancelota* (PL) swards as affected by different fertilization rates

Pasture (P)		LP			LP-PL			PL			*F/P*-value		
N rate	kg/ha	**0**	**150**	**300**	**0**	**150**	**300**	**0**	**150**	**300**	**P**	**N**	**P x N**
1st harvest	g	0.6^{aA} (0.02)	1.1^{aB} (0.2)	1.4^{aB} (0.1)	0.4^{abA} (0.02)	1.5^{aB} (0.1)	1.8^{aB} (0.1)	0.4^{bA} (0.04)	0.4^{bA} (0.1)	0.6^{bA} (0.1)	27***	88***	19***
2nd harvest	g	0.2^{A} (0.03)	1.8^{B} (0.3)	2.6^{B} (0.1)	0.3^{A} (0.1)	1.8^{B} (0.2)	2.7^{B} (0.9)	0.3^{A} (0.1)	1.5^{B} (0.4)	1.8^{AB} (0.2)	2^{ns}	62***	1^{ns}
3rd harvest	g	0.6^{A} (0.1)	4.5^{aB} (0.3)	4.6^{B} (0.6)	0.5^{A} (0.2)	4.0^{abB} (0.4)	5.0^{B} (0.1)	0.5^{A} (0.04)	2.6^{bB} (0.2)	4.3^{C} (0.3)	2^{ns}	247***	4*
Total harvest	g	1.3^{A} (0.1)	7.4^{B} (0.7)	8.6^{B} (0.6)	1.2^{A} (0.2)	7.3^{B} (0.6)	9.5^{B} (0.9)	1.2^{A} (0.1)	4.5^{B} (0.6)	6.6^{B} (0.3)	2^{ns}	259***	5**
Stubbles	g	0.8^{aA} (0.1)	2.3^{aB} (0.1)	2.9^{B} (0.4)	0.8^{aA} (0.1)	2.2^{aB} (0.1)	2.5^{B} (0.3)	0.4^{bA} (0.03)	1.2^{bB} (0.2)	1.6^{B} (0.1)	41***	151***	5**
AG DM	g	2.1^{bA} (0.1)	9.7^{bB} (0.8)	11.5^{B} (0.9)	2.0^{abA} (0.2)	9.5^{bB} (0.5)	11.9^{B} (1.0)	1.5^{aA} (0.1)	5.7^{aB} (0.7)	8.2^{C} (0.3)	23***	373***	1^{ns}
BG DM	g	1.5^{A} (0.2)	4.0^{B} (0.5)	4.6^{bB} (0.4)	1.4^{A} (0.2)	3.6^{B} (0.3)	4.6^{bB} (0.3)	0.8^{A} (0.1)	2.3^{B} (0.4)	2.2^{aB} (0.2)	21***	99***	4**
Root/shoot ratio	g/g	0.69^{A} (0.09)	0.42^{B} (0.03)	0.40^{aB} (0.04)	0.72^{A} (0.03)	0.39^{B} (0.05)	0.39^{aB} (0.03)	0.54^{A} (0.07)	0.41^{A} (0.03)	0.26^{bB} (0.03)	4*	29***	2^{ns}
Plant biomass$^{\alpha}$	g	3.6^{aA} (0.1)	13.7^{aB} (1.1)	16.1^{aB} (1.1)	3.4^{abA} (0.4)	13.1^{aB} (0.4)	16.5^{aB} (1.2)	2.4^{bA} (0.1)	8.0^{bB} (1.1)	10.4^{bB} (0.4)	18***	285***	7**
Species proportion	%	100	100	100	74.4/25.6	93.3/6.7	91.9/8.1	100	100	100	-	-	-
AG C/N ratio	g/g	29.6^{bB} (0.7)	31.7^{cB} (0.7)	22.1^{bA} (0.2)	31.4^{bB} (1.7)	25.2^{bA} (2.1)	22.3^{bA} (0.8)	20.1^{aB} (0.5)	19.6^{aB} (0.7)	15.0^{aA} (0.5)	95***	63***	4**
BG C/N ratio	g/g	44.6^{bAB} (3.1)	46.0^{A} (1.5)	39.6^{Bb} (1.1)	43.8^{ab} (2.0)	43.1 (1.3)	37.2^{b} (2.2)	35.8^{aAB} (0.8)	41.4^{B} (1.4)	29.5^{aA} (2.2)	13**	18***	2^{ns}
N uptake	kg ha^{-1}	16.7^{A} (0.2)	65.6^{B} (6.0)	107.0^{C} (6.7)	16.2^{A} (1.6)	74.5^{B} (2.0)	110.6^{C} (8.3)	15.9^{A} (0.8)	57.4^{B} (9.4)	104.4^{C} (3.7)	0.3ns	363***	0.9ns
SNUE	g g^{-1}	75.6^{bB} (2.2)	76.1^{cB} (1.9)	52.8^{bA} (0.5)	76.3^{bC} (4.1)	63.7^{bB} (4.4)	53.0^{bA} (2.0)	51.5^{aB} (1.6)	47.9^{aB} (1.8)	36.0^{aA} (1.0)	99***	70***	2^{ns}
RNUE	g g^{-1}	116.6^{b} (14.3)	123.5 (4.2)	103.9^{b} (1.3)	104.7^{ab} (3.6)	106.2 (2.7)	98.4^{b} (3.4)	88.9^{aA} (2.3)	115.4^{B} (4.9)	77.5^{aA} (5.6)	12***	13***	3*

LP represents *Lolium perenne; PL represents Plantago lanceolate.* SEM = standard error of the mean (pooled across pastures and fertilization rates; means with different letters are different at $p<0.05$, ^{abc} represent the effect of sward type, ^{ABC} represent the effect of fertilization rate. AG is above-ground biomass (harvested + stubbles); BG is below-ground biomass; Plant biomass$^{\alpha}$ is AG + BG.

Table 4.2: Cumulative N_2O emissions (83 days) as well as N_2O emission in relation to above- (AG) and belowground biomass (BG) among the different treatments

Pasture (P)	**LP**			**LP-PL**			**PL**			**F/P- value**		
N rate (N, kg ha^{-1})	**N0**	**N150**	**N300**	**N0**	**N150**	**N300**	**N0**	**N150**	**N300**	**P**	**N**	**P x N**
N_2O, kg N ha^{-1}	0.01^{A} (0.00)	0.09^{aA} (0.02)	0.55^{abB} (0.1)	0.01^{A} (0.00)	0.17^{abB} (0.04)	0.43^{aB} (0.1)	0.01^{A} (0.00)	0.24^{bB} (0.04)	0.85^{bC} (0.1)	0.09^{ns}	83^{***}	4^{**}
N_2O, g N kg BG^{-1}	0.01^{A} (0.00)	0.06^{aA} (0.01)	0.30^{aB} (0.04)	0.01 (0.00)	0.12^{ab} (0.04)	0.24^{a} (0.05)	0.02^{A} (0.01)	0.27^{bB} (0.09)	0.99^{bC} (0.15)	22^{***}	47^{***}	11^{***}
N_2O, g N kg (AG+BG)$^{-1}$	0.01^{A} (0.00)	0.02^{Aa} (0.00)	0.08^{Ba} (0.01)	0.01 (0.00)	0.03^{ab} (0.01)	0.07^{a} (0.02)	0.01^{A} (0.00)	0.08^{Bb} (0.02)	0.20^{Cb} (0.04)	2^{ns}	44^{***}	7^{**}
N_2O, g N kg N uptake^{-1}	0.41^{A} (0.12)	1.37^{aA} (0.13)	5.12^{abB} (0.5)	0.46^{A} (0.1)	2.30^{abB} (0.6)	4.06^{aB} (1.0)	0.49^{A} (0.17)	4.59^{bB} (1.5)	8.32^{bB} (1.6)	4^{*}	59^{***}	2^{ns}

Values in parenthesis are the standard error of the mean; means with different letters are different at $p<0.05$, ^{abc} represents the effect of sward type, ^{ABC} represents the effect of fertilization rate. *LP* represents *Lolium perenne; PL represents Plantago lanceolata*

The binary mixture achieved comparable yields as the *L. perenne* monoculture but was around 50% higher than those of the *P. lanceolata* monoculture independent of the fertilizer rate ($p<0.05$; Table 4.1). The root/shoot ratio effect was only evident at N300, where the *P. lanceolata* had a 32% lower root/shoot ratio than the *L. perenne*-treatment. The C/N ratio of the AG was significantly higher for *L. perenne* or the mixture compared to *P. lanceolata* at N0 and N300 and generally ranged between 15 and 32. The C/N ratio of the BG ranged between 30 and 46 and was 20% lower for *P. lanceolata* relative to *L. perenne* at 0N and 23% lower for *P. lanceolata* relative to the mixture or the *P. lanceolata* monoculture at the N300 rate (Table 4.1).

The proportion of *P. lanceolata* in the binary mixtures ranged between 7–26% (mean=13%), depending on the N rate and was negatively correlated with increasing N rate (Table 4.1). With increasing N fertilizer, the plant N uptake increased across all the treatments (Table 4.1). Compared with the control, the N300 rate reduced SNUE of the swards, but the N150 rate only decreased SNUE of the mixed swards. The N150 rate increased RNUE across swards compared with the control, although the differences were not significant for the *L. perenne*-rich swards (Table 4.1).

4.3.2 N_2O emission

Daily N_2O emission ranged from 0 - 8.70 mg N m^{-2} across all treatments, with means (±sd) of 0.01±0.02, 0.40±0.49 and 1.37±1.94 mg N m^{-2} at the N0, N150 and N300 rates, respectively. The highest peak fluxes of 0.11, 1.67 and 8.70 mg N m^{-2} d^{-1} observed for the N0, N150, and N300 rates, respectively, were recorded from the *P. lanceolata* swards (Figure 4.2). Generally, increasing N rates increased cumulative N_2O emission (~ 85-fold), but the differences were not significant in some cases (Table 4.2). For example, whereas the N300 rate consistently increased N_2O emission (kg ha^{-1}) across the swards compared with control, N addition at the N150 did not increase N_2O emissions from the *L. perenne* swards significantly ($p>0.05$; Table 4.2). Unlike the monocultures, the N150 and N300 rates did not affect ($p>0.05$) cumulative N_2O emission per pot, per root biomass or total biomass in the mixed swards (Table 4.2).

4.3.3 LAP expression

LAP expression in the soils increased after the first growth stage at all N rates and appeared to have stabilized or dropped slightly at the third growth stage (Figure 4.3a). Increasing N availability in the soils significantly ($p<0.05$) reduced LAP activity at each growth stage. LAP activity was highest in the mixtures and lowest in *P. lanceolata* monocultures ($p<0.05$), except for the N150, where differences between the two monocultures were marginal (Figure 4.3b).

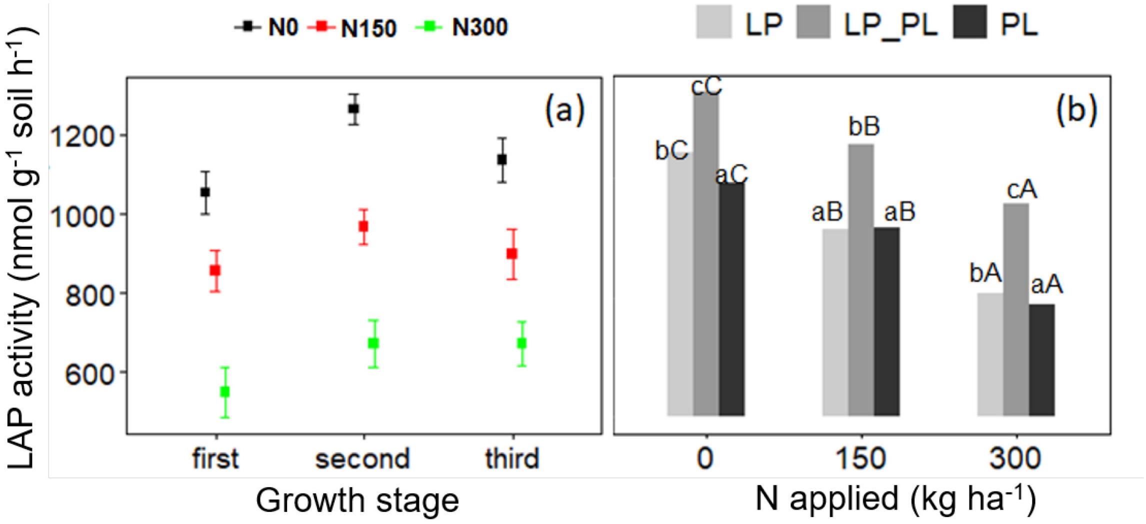


Figure 4.2: Graphs showing leucine aminopeptidase (LAP) expression in fertilized soils cultivated with forage species as affected by (a) stage of growth and (b) sward type. Lowercase letters represent significant differences between the sward types and uppercase letters between the N-rate within one treatment. LP represents *Lolium perenne;* PL *represents Plantago lanceolata*

4.3.4 Relationships between the measured variables

4.3.4.1 Binary relationships between N_2O emission and the plant/microbial traits

N_2O-N emission did not significantly correlate with any plant/microbial trait in the N0 treatment (Table 4.3). At N150, lower N_2O-N emissions were significantly ($p<0.05$) associated with a higher BG C/N ratio and higher SNUE; however, higher N_2O-N emission associated with higher *L lanceolata* proportions. Lower N_2O-N emission was associated with higher BG DM and higher microbial activity at the N300 rate (Table 4.3). Generally, a higher percentage of plantain in the sward correlated negatively with BG DM, BG C/N ratio and SNUE at all the N rates and BG C/N ratio correlated positively with SNUE at all the N rates.

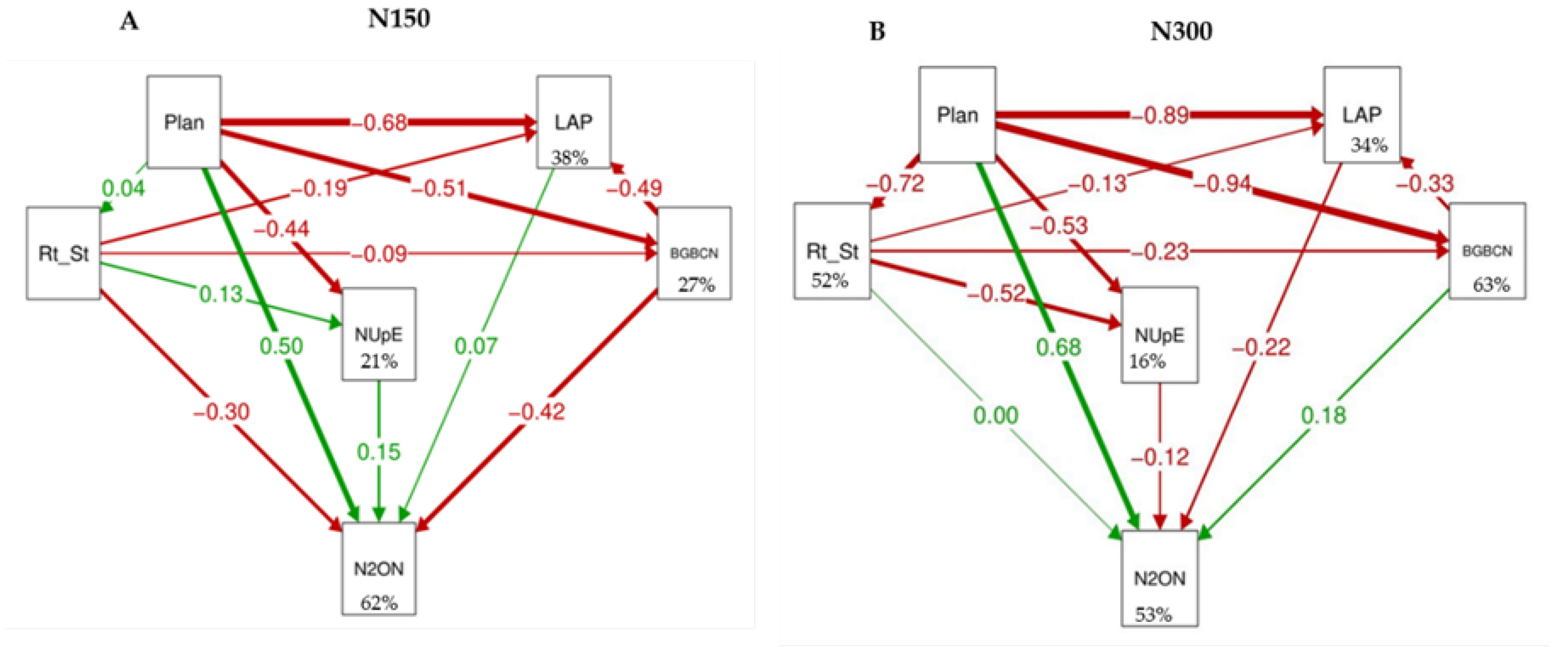
4.3.4.2 The relative importance of the plant/microbial traits to N_2O emission

The structural equation models (SEM) shown in Figure 4.3 describe the direct and indirect effects of the measured plant/microbial traits on N_2O emission. The SEM path coefficients largely agree with the correlation coefficients reported in Table 4.3. Accordingly, the N_2O emissions in the 150 N treatment were predominantly positively influenced by the percentage of plantain in the sward ($r=0.50$) and negatively influenced by the C/N ratio of the belowground biomass (*r*=-0.42) and the root/shoot ratio (*r*=-0.30), with all contributing factors resulting in a combined coefficient of determination of $R^2 = 0.62$ (Figure 4.3).

Table 4.3: Spearman correlation coefficients showing the relationships between N_2ON emission, biomass variables and microbial activity

	BG DM	AG C/N	BG C/N	NUpE	N_2ON	BG/AG	RNUE	SNUE	LAP	Plan
					N0					
AG DM	0.75^{**}	0.75^{**}	0.54	0.64^{*}	0.00	0.22	0.41	0.72^{*}	0.54	-0.78^{**}
BG DM		0.71^{*}	0.76^{**}	0.40	-0.28	0.78^{**}	0.78^{**}	0.64^{*}	0.53	-0.79^{**}
AG C/N			0.73^{**}	0.08	-0.09	0.29	0.57	0.95^{***}	0.75^{**}	-0.64^{*}
BG C/N				-0.01	0.06	0.54	0.93^{***}	0.74^{**}	0.50	-0.69^{*}
NUpE					0.11	0.15	0.01	0.03	-0.06	-0.40
N_2ON						-0.34	-0.15	0.00	-0.19	0.20
BG/AG							0.69^{*}	0.22	0.26	-0.44
RNUE								0.53	0.34	-0.61^{*}
SNUE									0.69^{*}	-0.73^{**}
LAP										-0.46
					N150					
AG DM	0.71^{*}	0.59^{*}	0.46	0.48	-0.49	-0.23	0.08	0.66^{*}	0.22	-0.73^{**}
BG DM		0.37	0.36	0.62^{*}	-0.56	0.47	0.31	0.53	0.16	-0.76^{**}
AG C/N			0.48	-0.30	-0.60^{*}	-0.18	0.28	0.95^{***}	-0.09	-0.81^{**}
BG C/N				0.15	-0.59^{*}	-0.11	0.62^{*}	0.52	-0.27	-0.65^{*}
NUpE					-0.03	0.20	-0.06	-0.15	0.23	-0.19
N_2ON						-0.19	-0.38	-0.73^{*}	-0.27	0.73^{**}
BG/AG							0.37	-0.08	-0.06	-0.15
RNUE								0.25	-0.62^{*}	-0.40
SNUE									0.10	-0.87^{***}
LAP										-0.04
					N300					
AG DM	0.83^{**}	0.80^{**}	0.69^{*}	0.66^{*}	-0.53	0.48	0.86^{***}	0.80^{**}	0.50	-0.60^{*}
BG DM		0.70^{*}	0.53	0.41	-0.60^{*}	0.84^{**}	0.82^{**}	0.71^{*}	0.59^{*}	-0.65^{*}
AB C/N			0.83^{**}	0.17	-0.52	0.50	0.89^{***}	0.99^{***}	0.58	-0.78^{**}
BG C/N				0.10	-0.57	0.38	0.81^{**}	0.83^{**}	0.41	-0.67^{*}
NUpE					-0.07	-0.07	0.36	0.19	-0.02	0.11
N_2ON						-0.54	-0.43	-0.52	-0.69^{*}	0.45
BG/AG							0.59^{*}	0.48	0.54	-0.72^{**}
RNUE								0.89^{***}	0.37	-0.75^{**}
SNUE									0.55	-0.79^{**}
LAP										-0.44

$^{\alpha}$Computed correlation used spearman-method with pairwise deletion. *BG* – below ground biomass; *AG* – aboveground biomass; DM– dry matter; *C*-carbon; *N*- nitrogen; RNUE/SNUE- root/shoot nitrogen use efficiency; NUpE- whole plant N uptake; *LAP*- leucine aminopeptidase, a proxy for microbial activity; level of significance: "***" $p<0.001$, "**" $p<0.01$, "*" $p<0.05$, " " $p>0.05$



$\chi^2 = 1.81$, $P = 0.40$, Df= 2
CFI= 1.00
TLI= 1.23
RMSEA= 0.00, CI90(0.000 – 0.555)
SRMR= 0.062

$\chi^2 = 0.27$, $P = 0.87$, Df= 2
CFI= 1.00
TLI= 1.58
RMSEA= 0.00, CI90(0.000 – 0.289)
SRMR= 0.024

Figure 4.3: Structural equation models showing the relationships between the measured variables at (A) N150 fertilizer rate and (B) at N300 fertilizer rate. Plan-forage plantain share; NUpE- whole plant N uptake; Rt_St- root/shoot ratio; BGCN-belowground biomass C/N ratio; LAP- leucine aminopeptidase, a proxy for microbial activity; green arrows represent a positive association, and red arrows represent negative association; numbers within arrows represent standardized path coefficients; whereby negative path coefficients indicate a negative relationship. The higher the absolute value of the path coefficient, the more important is the path. Numbers in the white boxes show the percentage of explained variance of each of the endogenous variables. X2 indicates the fit of the model, Comparative Fit Index (CFI), Tuckere Lewis Index (TLI), and the Root Mean Square Error of Approximation (RMSEA).

While the plantain share negatively influenced both the LAP activity (*r*= -0.68, R^2=0.38) and the whole plant N uptake (*r*= -0.44, R^2 =0.21), this did not translate into a strong influence on N_2O emissions. For 300N, the percentage of plantain in the biomass was again positively linked to the N_2O emissions (r= 0.68), with no other factors having strong direct impacts. However, again, the plantain percentage negatively impacted LAP activity (*r*= -0.89, R^2 = 0.34); this again did not translate into a strong impact on N_2O emissions (*r* = -0.22). At both N rates, plantain percentage and BG C/N ratio appeared to be the dominant factors influencing soil microbial activities. The plant and microbial factors' impact on N_2O emission reduced by about 10% when N application increased by 100%.

4.4 Discussion

4.4.1 Dry matter, carbon and nitrogen yields

Nitrogen application generally increased dry matter production, C and N yields (Appendix C Figure C1). This trend was expected as N is a significant component of chlorophyll required for plant metabolism, including proteins and photosynthates synthesis (OHYAMA, 2010). Nitrogen addition stimulates root growth and development, enhancing uptake of other nutrients (AI *et al.*, 2017). Application of N affected biomass yield most during the third growth stage. However, the *P. lanceolata* sward response to higher N rates was weaker than the *L. perenne*-rich swards. Surprisingly, DM yield did not differ significantly between the two fertilizer rates across the swards, despite a general tendency to increase yields (FERREIRA *et al.*, 2017). This was even though the N rates used in this study were within the range of annual inputs (up 450 kg N/ha) from fertilizer or fixation by legumes on most intensively cultivated pastures (BALL & RYDEN, 1984).

We hypothesized that the mixed sward would yield higher DM than the mono-species swards due to their higher capacity to utilize resources efficiently, even at the N300 rate (NYFELER *et al.,* 2009). Although DM yield was slightly higher (about 3%) for the mixed swards relative to the *L. perenne* swards, this difference was not statistically significant. The similar DM yield observed for mixed and *L. perenne* swards might be due to the relatively low proportion of *P. lanceolata* (average of about 13%) in the mixed swards, which probably did not suffice for optimal niche utilization. Regarding the monocultures, consistent with our observation, MINNEÉ *et al.* (2019) found a consistently lower (about 30%) DM yield from *P. lanceolata* than perennial ryegrass monocultures in a meta-analysis. This trend was attributable to the high water content of Plantago (O'CONNELL *et al*., 2016). This attribute of *P. lanceolata* can induce diuresis when consumed by livestock and has been described as beneficial as animals are required to increase their water uptake and dilute the concentration of N in the urine, thus minimizing N losses to the environment.

The results showed that N fertilization reduced the C/N ratio in both below- and aboveground biomass, with this trend being most evident in the N300 treatment. Thus, C and N were assimilated differently depending on the N rate, plant part or sward type (Table 4.1). Similarly, earlier authors reported increased N assimilation and decreased C/N ratios in maize straw (CHEN *et al.,* 2009) and grass swards (GORISSEN & COTRUFO, 2000; JIANG *et al.*, 2016) with fertilizer application. However, VAN GINKEL *et al*., (1997) observed no fertilizer effect on the root C/N ratio of *L. perenne* swards. Still, in most plants, nutrient availability and light intensity drive C acquisition and biomass allocation (IRVING, 2015). N supply is necessary to synthesise C and N assimilates (FOYER *et al*., 2001); thus, N is tightly linked with photosynthesis. The C/N ratio in plant parts is not fixed but varies according to the availability of resources, particularly N but also CO_2 (THEOBALD *et al.*, 1998). Inadequate N reduces amino acid concentration and decreases growth, leading to the accumulation of carbohydrates, hence a high C/N ratio (LAWLOR, 2002). On the other hand, higher NO_3^- uptake relative to CO_2 uptake increases amino acid accumulation relative to carbohydrates, leading to a low C/N ratio and growth stimulation (LAWLOR *et al*., 1987).

The N application also reduced the NUE of both above- and belowground biomass and was consistently lower for the *P. lanceolata* swards than *L. perenne* or mixed swards. The similarity between *L. perenne* and mixed swards in NUE might again be due to the high *L. perenne* proportion mixtures (Figure 4.1). NUE in the plant appears genetically determined (HEILMEIER & MONSON, 1994) and might differ from one plant to another. Significant uptake of N by plants depends, among other factors, on the efficiency of NO_3^- absorption by roots, which is a function of the storage capacity for NO_3^- (LAWLOR *et al.*, 1987). N availability promotes root growth, a direct response to increasing root capacity to accommodate the incoming NO_3^-. However, N accumulation reduces root sugar content, affecting plant growth as sugar content correlates with growth (SCHEIBLE *et al.*, 1997), partly explaining why at very high N application rates, NUE appears to diminish. As observed in this study, NUE significantly dropped when N was added at 300 kg ha^{-1} equivalent.

The proportion of *P. lanceolata* in the mixed-swards increased over time, particularly under low soil N conditions. This result was comparable to reports by (OLFF & BAKKER, 1991), who observed increased *Plantago* proportions in mixed swards on nutrient-poor soils, especially when the cutting intervals are extended. However, under the medium to high N fertilization, *P. lanceolata* proportions remained low, as a result of competition from perennial ryegrass in mixed swards. This trend was expected, as individual species' performance in mixtures depends on the companion species due to interspecific interactions, which can be synergistic or antagonistic. The strength of the interaction also depends on the species relative abundances (BLACK *et al*., 2017). Perennial ryegrass is a fast and aggressive species and has a high capacity to utilize N for biomass productivity, as shown in this study and might dominate mixed plant communities (ABALOS *et al*., 2014). This attribute of *L. perenne* swards might partly explain the higher response of the *L. perenne* swards to N fertilization than the *P. lanceolata* swards.

4.4.2 LAP activity

One of our objectives was to assess whether changes in soil N status and differences in sward diversity affect LAP enzyme activity. The swards' structural and functional differences affected LAP expression in the soils, with a higher enzyme activity associated with the mixed swards relative to the monocultures at all N fertilization rates. Plants may affect the abundance and activity of soil microbes, especially those involved in N cycling. Previous studies reported high microbial activity associating with more acquisitive swards than conservative swards (BAXENDALE *et al*., 2014; GRIGULIS *et al*., 2013; ORWIN *et al*., 2010). This trend might relate to the high exudation associated with mixed-species communities and the consequent stimulation of microbial activity. Soil microorganisms derive energy from root exudates for biomass production (BAUDOIN *et al*., 2003). The amount and type of exudates depend on several factors, including the plant species, plant age (HERTENBERGER *et al*., 2002; MARTIN & BREWBAKER, 1971) and soil factors (e.g. soil type, moisture; (BOONE *et al*., 1998; NEUMANN *et al*., 2014). Accordingly, plants often affect their rhizobiomes via root exudation (HOOPER *et al*., 2000, MOMMER *et al*., 2016). Variations and stoichiometry in root exudates can alter microbial communities and functions and influence the relationship between plant diversity and microbial communities (HOOPER *et al*., 2000).

Plant-soil- feedbacks might determine soil biota and enzyme expression changes (BEVER *et al*., 2012). ALLISON *et al.* (2011) hypothesized that extracellular enzyme production is under evolutionary pressure to minimize enzyme production's cost-benefit ratio. Under low N soil conditions, plants depend heavily on microbes to acquire N. This extra demand from the plants stimulates microbes to express N-acquiring enzymes to mine N (LIU *et al.*, 2018). Thus, LAP activity in soils may be a good indicator for evaluating N cycling response to N availability in soils (SHI *et al.*, 2016). However, LAP expression response to N availability might be mediated by environmental conditions, including soil pH, soil water availability, and enzyme co-factors availability (SHI *et al.*, 2016). Although we did not measure soil pH changes in this study, it is speculated that N addition must have reduced soil pH (BARDGETT *et al.*, 1999; BARNEZE *et al.*, 2020; YOU *et al.*, 2017). N addition is also associated with increased biomass production (Table 4.3), which increases leaf transpiration, leading to water loss (XU *et al.*, 2010; ZITER & MACDOUGALL, 2013). Thus, the possible decreasing soil pH and reducing water availability due to increasing N addition may negatively affect microbial activity, and for that matter, LAP expression (DICK *et al.*, 2000; ZHOU *et al.*, 2013).

Furthermore, we found a positive association between LAP expression and the BG C/N ratio (Table 4.3; Figure 4.3). Similarly, previous authors (EISENHAUER *et al.*, 2017; THAKUR *et al.*, 2015) reported a positive association between increased root biomass, root exudates and microbial biomass as well as increased species richness. PROMMER *et al.* (2020) reported a direct relationship between root biomass, microbial growth and microbial biomass carbon. Thus, higher C availability below ground will stimulate microbial growth (HOPKINS *et al.,* 2014) and further explain why there appeared to be an increased LAP activity associated with increasing growth stage and the mixed swards (Figure 4.2). However, soil microbial communities can also affect root exudates' amount and composition (MEIER *et al.*, 2013). SØRENSEN *et al.* (2009) contended that various plants' different sets of organic compounds affect the structure, abundance, and microbes' activity. Thus, more diverse exudates would stimulate a higher microbial diversity (PHILIPPOT *et al.*, 2013) and alter extracellular enzyme expression (KREYLING *et al.*, 2008). Moreover, we found increasing N availability associated with decreasing LAP expression (Figure 4.3). This trend was also reported for temperate Mongolian steppe (SHI *et al.*, 2016) and other grassland ecosystems (CENINI *et al.*, 2016; STURSOVA *et al.*, 2006).

4.4.3 Nitrous oxide emission

Nitrogen balance increased with increasing N rate applied, consequently increasing N_2O emission from the swards. Increasing rates of N supply were expected to increase N_2O emission as N_2O emission is a function of soil residual N. The oxidation of NH_4 and reduction of NO_3 results in N_2O production and consumption in terrestrial ecosystems. N fertilizers provide a rich source of N for the nitrification and denitrification processes by microbes (ALLEN *et al.*, 2010; CAI *et al.*, 1997; SUN *et al.*, 2016), given that all environmental conditions are suitable. NH_4 forming fertilizers (urea in this case) undergo rapid nitrification to stimulate N_2O production. Generally, fertilizer application increases N_2O emissions for approximately 5-8 weeks (BOUWMAN *et al.*, 2002; GRANLI & BØCKMAN, 1995; MÜLLER & SHERLOCK, 2004). Hence, the consequent effect of N addition on N_2O emission observed in this study is not surprising.

However, it was previously reported that *P. lanceolata* may release plant secondary metabolites, including mucilage, pectin, flavonoids and tannins, which inhibit microbial growth (PENSANTES-SANGAY *et al.*, 2020). The release of these chemicals via root exudates was reported to inhibit nitrification in soils (CARLTON *et al.*, 2019; GARDINER *et al.*, 2018; JUDSON *et al.*, 2019; MANGWE *et al.*, 2019). Thus, the negative relationship between the *P. lanceolata* share in the swards and LAP expression observed in this study (Figure 4.3) is not surprising. We hypothesized that a higher *P. lanceolata* proportion in the swards might reduce N_2O emissions from the soils. Contrary to this, a higher *P. lanceolata* proportion in the swards resulted in higher N_2O emission (Table 4.3; Figure 4.3). This observation is consistent with previous studies, which reported no difference in N_2O emission or the growth of ammonium-oxidizing bacteria (AOB) when ruminant urine N was applied to ryegrass-white clover pasture and pastures containing 30–100 % plantain (PODOLYAN *et al.*, 2020) and there was no evidence of plantain affecting soil inorganic-N concentrations via biological nitrification inhibition following ruminant urine N application (LUO *et al.*, 2018; ROSEAUX *et al.*, 2020; SIMON *et al.*, 2019).

In contrast, (CARLTON *et al.*, 2019) reported reduced AOB abundance for a *P. lanceolata* rich pasture (ca. 30% plantain), with a strong impact of contrasting soils and environmental conditions. The presence of a symbiotic association of plants with arbuscular mycorrhizal fungi (AMF) is known to affect N cycling by reducing available N, with consequent effects on denitrifying and nitrifying communities and N_2O emission (BENDER *et al.*, 2014; HODGE & FITTER, 2010; VERESOGLOU *et al.*, 2012). Meanwhile, the abundance of AMF is reported to vary with plant age, with well-established AMF communities reducing N_2O emission significantly (VERESOGLOU *et al.*, 2012). Thus, it appears this age effect might explain the lack of a plantain nitrification inhibition effect in the current study and previous mesocosm experiments (PIJLMAN *et al.*, 2020; ROSEAUX *et al.*, 2020), which involved less established PL swards when compared with the experiment by CARLTON *et al.* (2019) which involved older PL pastures (about 2 yrs). Besides, the NI effect of forage plantain might depend on other ecological factors, including light irradiation, soil fertility, water, salinity, temperature, microbial degradation, or interaction with soil particles (YANG *et al.*, 2018; LUO *et al.*, 2018).

Previous authors have suggested the use of yield-scaled N_2O emission or N_2O emission intensity (cumulative N_2O divided by biomass or N uptake; (VAN GROENIGEN *et al.*, 2010) to identify potential trade-offs between food production and environmental sustainability (PITTELKOW *et al.*, 2014; ABALOS *et al.*, 2018). However, yield-scaled N_2O emissions showed a similar trend, suggesting the *L. perenne*-based pastures' superiority in ecological efficiency. At low soil N conditions, N_2O emission and emission intensity were similar across the forage swards but differed at higher soil N concentrations, suggesting that grasslands' response to N_2O emission depends on soil N status. Root/shoot and biomass C/N ratios were higher for *L. perenne*-rich swards than the *P. lanceolata* swards, and higher root/shoot ratio or higher belowground C/N ratio were associated with low N_2O emission. Furthermore, biomass allocation belowground correlated positively with a high BG C/N ratio as well as microbial activity (Figure 4.3; Table 4.3). These results point to the assertion that the C belowground availability stimulates microbial activity, including boosting N consuming species' activity. This relatively enhanced microbial activity must have resulted in the consumption or immobilization of soil residual N (BIERNAT *et al.*, 2020; DE KLEIN *et al.*, 2019). From the results, it can be hypothesized that the relatively high N_2O emission observed from the *P. lanceolata* swards compared with the *L. perenne*-rich swards might be due to the lower resource allocation belowground, which limited their capacity to store N and limited the activity N- acquiring microbes. Meanwhile, the negative relationship between N_2O emission and microbial activity at the N300 rate projects N-acquiring microbes' role as N_2O-N consumers. Thus, a higher abundance of N_2O consuming microbes might lead to reduced N_2O emission.

Root/shoot and biomass C/N ratio were slightly higher for the *L. perenne* swards than the mixed swards; however, N uptake, N uptake efficiency and LAP expression were 6 %, 9% and 41%, respectively, higher, while N_2O-N emission was 9 % lower for mixed swards compared with the *L. perenne* swards. Previous studies have reported the high N use efficiency of mixed swards due to their high potential for niche differentiation and species complementarity (NYFELER *et al.*, 2009). Moreover, the high microbial enzyme expression associated with the mixed swards compared with the mono-crops might be due to increased root exudation, which must have stimulated the proliferation of N-acquiring microbes. However, the abundance of N-acquiring microbes, such as AMF, in soil declines with fertilization and intensive land use (Figure 4.2b; EGERTON-WARBURTON & ALLEN, 2000; OEHL *et al*., 2004), which consequently reduces the N_2O mitigation potential of forage species (Figure 4.3). These results suggest that low input systems may have a higher potential to enhance beneficial plant-microbe symbiotic associations with positive consequences for the ozone layer and the earth's climate. The high association between LAP expression, root/shoot ratio, and N_2O emission suggests that LAP is a potential biomarker for screening pastures for low N_2O emissions.

4.5 Conclusion

This study aimed to understand the role of plant belowground resource allocation in N_2O emission and establish the potential impact of forage plantain on N_2O emission from soils of northern Germany. N addition increased biomass C and N, which was consistently higher for the *L. perenne*-rich swards than *P. lanceolata* monocultures. Contrary to our hypothesis, higher *P. lanceolata* proportions in the swards increased N_2O emission but reduced N-acquiring microbes' activities. The mixed swards saw the most increased microbial activity and the highest NUE, suggesting LAP activity as a potential indicator or biomarker for higher efficiency. Besides, the results suggest C allocation dynamics belowground as a likely means by which plants impact N_2O emission. Forage plant breeders could explore this to enhance the capacity of intensively cultivated grasslands to mitigate N losses to the environment. Our study highlights the need to factor plant traits into models that predict N_2O emission from soils.

CHAPTER 5

General discussion

5.1 Forage plants and the livestock- climate change nexus

Agriculture is an essential anthropogenic activity that predates modern history, and its importance keeps growing through transformations to meet the growing demand for food. However, the impact of agriculture on the environment has become a sharp focus, especially as future demand for food is projected to double over the next 2-3 decades. The negative impact of agriculture on the environment is primarily blamed on intensification and simplification of production evident on the field, farm, landscape or regional scale (LEMAIRE *et al.*, 2014). Grassland use contributes to climate change and environmental degradation but can also be used as a tool for greenhouse gas emissions mitigation. Most low-input systems, spanning from extensive mixed farming to organic farming, which form a substantial part of the global agricultural production systems, are located in grasslands. These systems must be optimized to increase both production and ecological efficiencies to remain sustainable. Optimizing production will require the optimization and use of internal production inputs (i.e., on-farm resources) to lower production costs, avoid pollution, reduce the farmer's overall risk, and increase both short and long-term farm profitability (PARR *et al.*, 1990).

Low-input systems are often considered ecologically beneficial; however, reducing inputs might either reduce or increase environmental impacts per product unit, depending on the initial situation and the impacts under consideration (KULAK *et al.*, 2013). Assessing the ecological efficiency of farming systems requires appropriate data upon which production decisions could be made. However, data on agroecological issues are scarce, particularly from developing countries where research investment is usually low (HARRIS, 2004). Due to data inadequacy, generalized proxies or models are used to estimate, for example, GHG emissions at the landscape, regional or global scales. GHG emissions from agriculture are already associated with high uncertainties because processes leading to greenhouse gas production are susceptible to environmental factors (FIRESTONE & DAVIDSON, 1989; FLECHARD *et al.*, 2007; IPCC, 2006; VAN DER WEERDEN *et al.*, 2020; VOGLMEIER *et al.*, 2019). Much more data covering gradients of environments and management practices are required to reduce the colossal uncertainty associated with greenhouse gas production. Apart from presenting actual measured figures regarding SOC stocks and N_2O emission and emission factors from the case studies, this thesis also deepened our understanding regarding factors that might affect GHG emissions from such a neglected food basket. The results from these studies would generally contribute towards the attainment of global goals of environmental protection as seen in various treaties and conventions such as the "4 per mille" concept adopted at the 21st Conference of the Parties to the United Nations Framework Convention on Climate Change in Paris (MINASNY *et al.*, 2017), the Sustainable Development Goals (e.g. SDG15; UNCCD, 2015) and many other regional and national programmes.

Adoption of improved grassland species is expected to improve productivity while offering ecological services. Therefore, there is strong advocacy for using diverse grasslands to impact soil health, fertility, and productivity while delivering environmental services (DE KLEIN *et al.*, 2019; SUTER *et al.*, 2015). However, pasture species' role as greenhouse gas mitigation agents is poorly researched (BARNEZE *et al.*, 2020; FUCHS *et al.*, 2020). Interactions among plants or between plants and soil within an ecosystem result in feedback systems that regulate ecosystem functions, thus, making grassland species composition an essential factor (BARDGETT, 2011; BARNEZE *et al.*, 2020; DE DEYN *et al.*, 2008; LOREAU & HECTOR, 2001; TILMAN, 2001). However, the effects of grassland species composition on greenhouse gas (GHG) emissions is relatively unexplored (ABALOS *et al.*, 2014, 2018; BARNEZE *et al.*, 2020; SUN *et al.*, 2013). Although the past few years have seen increasing research to ascertain the potential of grasslands in enhancing ecological efficiency within farms, several issues remain unresolved, three of which are addressed in this thesis: (i) the prospects of low input forage production systems regarding C and N emission mitigation remain blurred, (ii) the factors that might confound the emission mitigation potential of low-input forage systems are not well understood, and (iii) the impact of mixed-sward pastures, particularly those containing forage plantain on N losses, has not been consistent. This thesis explored these issues to ascertain the degree to which existing forage species/pastures can enhance the ecological efficiency of low-input ruminant livestock feeding systems.

5.2 To what extent do existing forage species affect C and N cycling under low-input management systems?

In this thesis, the forage species' botanical and functional diversity at the farm scale significantly influenced SOC storage (*Chapter* 2) and N_2O emission (*Chapter* 3 & 4). The wide variation in SOC stocks (range = 16–42 Mg ha; CV=27–30%) among species within each functional group suggests that some of the forage species have unique characteristics, leading to a differential impact on SOC storage and soil chemical properties. Among the host of plants encountered (*Chapter* 2), however, only a few grass species (such as *Cenchrus ciliaris, Tripsacum laxum, Pennisetum purperuem*) and legume tree/shrubs (such as *Baphia nitida, Cajanus cajan and Gliricidia sepium*) appeared to have a higher potential (*Chapter* 2) to impact the soils positively under the current management practices (Appendix Figure A1). The tropical herbaceous legumes encountered in this study (*Chapter* 2) had relatively low SOC storage potential (*Chapter* 2). However, when established in mixtures with grasses, the tropical legume herbaceous species could improve soil fertility, belowground NPP and consequently SOC storage capacity (LÜSCHER *et al*., 2014; MORTENSON *et al*., 2004; RUZ-JEREZ *et al*., 1994).

It was found that diversity among the mixed pastures did not affect annual N_2O emission significantly both under grazing and simulated grazing (*Chapter* 3), contrary to our hypothesis. Nevertheless, a decreasing cumulative N_2O emission was evident when excreta N_2O emitted was related to the N uptake. Although the role of belowground spatial complementarity is not well understood (HOEKSTRA *et al*., 2015), it appears one main route by which mixed swards affect N_2O emission is by their high N uptake efficiency (*Chapter* 4). However, the efficiency with which such pastures take up N from the soil could be limited by unfavourable soil moisture conditions, which also have implications for N_2O emission (*Chapter* 3). In *Chapter* 3, for instance, there was a decreasing trend of N_2O emission with increasing grassland diversity from grazed plots in 2019 but not in 2018. The mean soil temperatures for the two years were similar, but rainfall was slightly higher in 2019 compared with 2018. The relatively higher soil moisture conditions in 2019 might have enhanced the diverse pastures' capacity to impact N use from the soils. This hypothesis could be tested further to understand better the factors that might confound the N use efficiency of diverse pastures.

The effect of legume proportions in mixed swards on N_2O emission is not well understood (BARNEZE *et al*., 2020; FUCHS *et al*., 2020). This thesis (*Chapter* 3) showed that higher legume share in swards might increase N_2O emission by increasing soil residual N. However, N_2O emission from the legume-based pastures was relatively low from both grazed and simulated plots on the field (*Chapter* 3). This trend is attributable to high productivity and high N assimilation into aboveground biomass (BARNEZE *et al*., 2020; FUCHS *et al*., 2020). Moreover, higher legume proportions in pastures might decrease ecosystem respiration in fertilized pastures, leading to C and N accumulation in soils (DE DEYN *et al*., 2011) as well as in unfertilized systems due to the increase of above- and belowground biomass, with positive implications for C and N storage in the soil (WU *et al*., 2017).

Many forage species, including legumes, herbs, and grasses, contain bioactive chemicals with environmental benefits (DE KLEIN *et al.*, 2019; VERMA *et al.*, 2021). This thesis has shown that topical forage dicots yielding more than 200kg CT/ha/year might positively affect SOC storage in the tropical grassland (*Chapter* 2). The role of plant secondary metabolites in SOC stabilization and mean retention time extension has been reported in the literature (ADAMCZYK *et al.*, 2017; CHOMEL *et al.*, 2016; HALVORSON *et al.*, 2011; KRAUS *et al.*, 2003; TAMURA & THARAYIL, 2014). The CT levels in the dicots at the site were within acceptable livestock feeding levels (*Chapter* 2). Thus, the additional ecological benefit from the tree/shrub species in this agroecosystem is essential, particularly as CTs are already beneficial in animal nutrition, with appreciable levels being able to reduce enteric methane emission, improve amino acid supply, act as an anthelminthic and reduce urinary N excretion (NYAMEASEM *et al.*, 2017; VERMA *et al.*, 2021).

The mechanisms through which individual plants directly impact N_2O emission is not well understood. DE KLEIN *et al.* (2019) suggested that a key mechanism by which plants such as plantain affect N_2O emissions is through root exudates that inhibit nitrification and/or increase available C and thus N immobilization. In *Chapter* 3, the presence of forage plantain in the most diverse pasture, a species known to be rich in bioactive compounds and effective against nitrification (SIMON *et al.*, 2019), did not impact N_2O emission or N_2O emission factor. In an earlier study (PODOLYAN *et al.*, 2020), the N_2O emission mitigation effect of forage plantain was only evident at 30-100%. Therefore, it was assumed that the most diverse pasture (*Chapter* 3) did not affect N_2O emission, probably due to the low forage plantain percentage in the mixture. Further work was necessary to establish the efficacy of forage plantain as a valuable plant to mitigate N_2O emission from pastures under northern Germany's environmental conditions.

As reported in *Chapter* 4, results showed that accumulated N_2O emission was about two times higher for the 100% forage plantain swards than the perennial ryegrass-rich swards, although the inhibitory effect on microbial activity was evident. The result is similar to previous reports, which also involved young plantain swards (Pijlman et al. 2020; Roseaux et al. 2020) but in contrast with reports by Carlton et al. (2019), who used relatively older pastures (about 2 yr old). These results suggest the probable effect of sward age on plantain’s ability to inhibit nitrification. At the same time, environmental factors must have constrained the forage plantain's bioactivity (YANG *et al.*, 2018). Meanwhile, further studies to identify such factors might inform breeding decisions towards improving this promising forage species' potentials. However, in this thesis (*Chapter* 4), it was shown that the allocation of more biomass C belowground reduced N_2O emission. It appears that higher biomass allocation belowground enhanced the N uptake efficiency of roots and increased microbial activity, leading to immobilization of N. This proposition could be explored further to improve forage accessions in that regard. It is worth mentioning that higher belowground biomass allocation may also enhance pastures' SOC storage capacity (WILSON *et al*., 2018).

5.3 How could the capacity of pastures be enhanced within the farm system to mitigate GHG emissions?

At the farm scale, land use/management differences resulted in varying SOC stocks (*Chapter* 2) and N_2O emissions (*Chapter* 3). The conversion of native grassland to arable cropping or forage monocultures resulted in SOC losses, except for shrubby/tree legume fodders. Carbon storage in soils is determined by the balance of inputs and outputs in respiration, caused mainly by soil disturbances (SOUSSANA *et al*., 2010). Conversion of native grasslands to agriculture within the first few years results in SOC losses due to the soil's disturbance, exposing stored C to decomposition and losses by respiration (DENG *et al*., 2016; SOUSSANA *et al*., 2010). However, the management style adopted after land conversion might influence the soils' SOC storage potential. Management decisions might affect the soil's physical, chemical, and biological properties, increasing or decreasing organic C content (LAL, 2004; WANG *et al*., 2018). Farm practices that increase organic matter input or reduce outputs promote C sequestration in the soil (THE ROYAL SOCIETY, 2018). SOC storage in low-input systems might be enhanced by increasing biomass input (either by applying farmyard manure or by adjusting harvested biomass), decreasing losses from erosion or decomposition (through cover cropping or mulching) or increasing mean retention time by stabilizing the sequestered SOC through minimal tillage (LAL, 2018). Other feasible measures might include an appropriate combination of eco-efficient genotypes to minimize nitrification rates or reduce urinary N excretion. Further, the grass, legume, and herb species' potential could be explored to obtain species ratios that optimize N uptake and minimize economic and environmental losses. The implementation of the strategies mentioned above could be complicated and might require further field studies and modelling.

Grazing is a significant source of methane (CH_4), nitrous oxide (N_2O) and carbon dioxide (CO_2) emissions. Moreover, grazing instead of cut –and –carry system increased N emissions (*Chapter* 3) via the return of high loads of feed N (SELBIE *et al*., 2015). Meanwhile, grazed livestock production in Ghana's grassland ecosystems is an integral part of the cultural, social, and economic identities of communities. However, grazing has been shown to stimulate C sequestration in soils with inconsistent results (MCSHERRY & RITCHIE, 2013). This study (*Chapter* 2) found the lowest SOC stocks in the grazed paddocks, probably due to improper grazing management and frequent pasture renovation (REINSCH *et al.*, 2018). On the contrary, light grazing instead of heavy grazing, converting grass leys to grass-legume mixtures and avoiding frequent tillage could enhance SOC storage at the paddocks (SOUSSANA *et al.*, 2010). These actions could increase N or organic matter input and decrease the decomposition of SOC. Grazing could be optimized to improve N uptake further at the site by adopting appropriate animal, plant and soil management practices on the grazing fields. These measures might include the appropriate timing of grazing to regulate N input and maximize N uptake or minimize N losses.

In *Chapter* 2, it was shown that soil nutrient availability, especially N, was a critical driver of SOC stocks in the low-input forage systems of SSA. This strong N effect and its interaction with P and/or K show the importance of fertilizer application if substantial SOC sequestration is to be achieved in this agroecosystem. Replenishing lost soil nutrients enhances belowground NPP, the primary means by which pastoral systems increase SOC sequestration. However, increasing the N fertilizer supply may not be ideal, as it promotes the expansion of grasses over dicots in mixed pastures. Moreover, the abundance of N-acquiring microbes, such as AMF, which positively influence the N cycle, might decline with increased N fertilization, decreasing the N_2O mitigation potential of forage species (*Chapter* 4). Accordingly, low input systems may have a higher potential to enhance beneficial plant-microbe symbiotic associations with positive consequences for the ozone layer and the earth's climate.

The results also suggest that species that use soil nutrients optimally might impact SOC sequestration in the low-input system. It, therefore, suffice to suggest that efforts be geared towards identifying or breeding plants with high N use efficiency, as N is always a limiting nutrient in such systems (PASLEY *et al.*, 2019). Also, plants with high N uptake efficiency had reduced N losses (*Chapter* 3 & 4). However, higher residual N (or surplus N) in soils might result in N losses to the environment (*Chapter* 3 & 4). By extension, it could be said that plants with relatively high N uptake efficiency might have a higher potential to mitigate C and N losses from low-input agroecosystems. In this regard, adopting mixed-sward systems, including legumes species, might enhance C sequestration in these soils (LANGE *et al.*, 2015). The inclusion of forbs and N-fixing legumes in mixed-pastures is imperative, particularly as these functional groups are more responsive to elevated CO_2 levels (BACA CABRERA *et al.*, 2021).

Irrigation could serve as an essential strategy to mitigate GHG emissions by enhancing pastures' productivity, leading to an increased grassland capacity to assimilate more N into biomass. In northern Germany, this might be necessary for later spring and summer but will require that soil moisture is just enough to meet plant needs as excess soil moisture might enhance N losses to the environment via denitrification or leaching. As reported in *Chapter* 3, relatively high water-filled pore space (about 60%) almost doubled N_2O emission from a binary pasture.

5.4 Some methodological perspectives

To obtain credible data, the methods by which the data is obtained and handled is essential. The methods adopted in this thesis generally followed recommended procedures (BUTTERBACH-BAHL *et al.*, 2016; IPCC, 2006, 2019). However, SOC sequestration assessment is often constrained by spatial and temporal variability, thus requiring proper sampling techniques and long-term field experiments (lasting several years and decades) to detect changes in C stocks (NAYAK *et al.*, 2019). It must be noted that C sequestration in agricultural systems can be determined over a relatively shorter period using the Edy-covariance flux system, which can measure net ecosystem exchange (NEE), net ecosystem production (NEP) and carbon sequestration but at a landscape or whole-farm scale (CHAPIN *et al.*, 2006; POYDA *et al.*, 2020). Another emerging method of measuring SOC sequestration is through the life cycle analysis (LCA) approach at a farm or even larger scales (GOGLIO *et al.*, 2015; KIMMING *et al.*, 2011; NAYAK *et al.*, 2019). The fields/plots used in the SSA study (*Chapter* 2) were about 50 years old under almost the same management conditions but were not originally designed to address the objectives set out in this thesis. As a result, the sample sizes of the experimental units were unbalanced. This irregularity was, however, resolved using appropriate statistical procedures to produce fair results. The sampling methods adopted in this thesis (*Chapter* 2) were most appropriate for plot/paddock scale SOC measurements, with the added advantage of being cheap and easy to apply in such a system.

The determination of initial SOC stocks to assess SOC stock changes is imperative in C sequestration studies (OLSON, 2013). In this study (*Chapter* 2), data on initial SOC stocks could not be obtained. This difficulty was especially so as the experimental fields/plots were not initially set up for this study. Consequently, SOC stocks from the converted land-use types were compared with the unconverted (non-treated) native grassland as the baseline (FRANZLUEBBERS, 2010; NAYAK *et al.*, 2019; SHAHID *et al.*, 2017) without reporting the initial SOC level (before treatment is implemented). This procedure might not accurately detect SOC change, steady-state, retention, and SOC loss within the baseline soil (OLSON, 2013). Moreover, the depth of soil sampling is vital as C concentration is a function of soil sampling depth (WUEST, 2009). This thesis considered SOC concentration in the top 30 cm depth, which is the usual soil depth recommended by the (IPCC, 2006), to facilitate comparisons across other agroecosystems. However, this depth might fail to capture the full effect of deep-rooted species encountered at this site (tree/shrub species) and might have underestimated the SOC stocks reported for the tree/shrub species in *Chapter* 2. Deeper depths are, therefore, recommended for such forage systems to uncover their full potential.

Gas sampling from both the simulated and non-simulated experiments and N_2O quantifying were done using the static chamber and gas chromatography methods, respectively. Although these traditional methods are characterized by poor spatial and temporal resolution, they are more flexible in applying at a smaller scale with relatively lower costs. Micrometeorological methods, including the Eddy covariance method (a laser technology), have been used to measure N_2O emissions from grazed pastures (VOGLMEIER *et al.*, 2019; WECKING, 2021). These methods offer continuous measurements but are relatively expensive and can only be used at a much larger scale, with frequently changing wind direction or low wind speed being some factors that might challenge their use. Thus, the chamber method was chosen to capture the magnitude and temporal patterns from emission hotspots (i.e., urine and dung patches) in this thesis (*Chapter* 3) due to its adaptability to plot scale measurements, better control of treatments and the possibility to compare the different treatments in parallel. In addition, the accuracy of measurements was expected to be enhanced with an increased interval of measurements (BUTTERBACH-BAHL *et al.*, 2016). The simulated experiment with controlled urine and dung depositions allowed for more uniform N loadings and tracking of emissions from exactly one urine or dung patch. Chambers effectively identifies processes and treatment effects and mitigation; however, more versatile high frequency and sensitivity sensors should be the future focus (HENSEN *et al.*, 2013).

5.5 Outlook

This thesis has clarified the actual C storage under long-term low-input land use in a tropical ecosystem. However, the measurements cannot be compared across different soils or climatic conditions due to the lack of similar long-use sites in the sub-region. Therefore, while the values presented in this study provide estimates upon which management decisions can be taken, care must be taken when comparing the results with similar land-use types under different soils or ecosystems. Moreover, the sample sizes used in *Chapter* 2 were restricted as some plots/fields had to be removed for non-compliance with the set criteria. As a result, the samples sizes were unbalanced, with a relatively high sample size for grass compared with the other functional groups. Thus, care must be taken when comparing the potentials of the plant functional groups. Again, it was not possible to capture the C storage potential of the individual species encountered in the SSA study, as many of the plots lacked replication, though the use of PCA revealed some potential species (Appendix A, Figure A1).

To improve the characterization of the ecological efficiency of the land-use types and forage species in the grasslands of southern Ghana, comprehensive longitudinal studies covering a range of soils, climatic zones and forage production systems will be worthwhile. Locating land use types under a relatively long-term uniform usage might not be possible. However, net ecosystem respiration experiments could be used to estimate potential C storage and GHG emissions. A comprehensive study of selected tropical species, especially those identified as having a relatively high beneficial effect on the environment, and mixed swards, covering their nutritive value, botanical traits, C and N assimilation above- and belowground and GHG emission could be beneficial. In considering CT effects on SOC storage, leaf/stalk CTs were used for the assessment. While this provided a general understanding of CTs' potential role on SOC storage in tropical soils, the effect size could not be estimated as polyphenol content in root might not be similar to shoots. Further studies might be necessary to quantify the effect of belowground polyphenol content on SOC storage in different soil types.

Grazing has been shown to stimulate SOC storage in grasslands when well managed. In this thesis (*Chapter* 2), grazed paddocks had the lowest SOC stocks. A further study to identify factors that might affect C sequestration in grazed pastures might be helpful. This study must, however, cover different soils and climatic zones within the tropical grassland ecosystem. Information from such a study could help formulate strategies to enhance grazing benefits in the sub-region.

In *Chapter* 3, the N_2O emission factors for urine and dung were estimated based on measurements spanning one year. Care must be taken when using the values in calculations owing to inter-annual variability in climatic conditions. Again, N_2O emission is highly dependent on N input, and N loads depend on grazing intensity. To improve the N_2O EF values, further measurements over several years covering different soils, pastures, and climatic zones will provide data that could develop models for estimating N_2O emissions across northern Germany. Moreover, forage plantain share in perennial ryegrass mixed sward dropped temporally, especially under N fertilization, probably due to perennial ryegrass's aggressive nature under high soil N availability (*Chapter* 4). Therefore, a higher plantain percentage in the mixed swards might have a different effect on N_2O emission than presented in *Chapter* 4. Experiments to determine appropriate seed rates to maintain an appreciable plantain share in mixtures will be worthwhile, with possible effects on urinary N concentration and N_2O production in soils. Another issue is that plant bioactive compounds act differently under different environmental conditions. In *Chapter* 4, the plantain effect on N_2O emission mitigation was not evident under greenhouse conditions but might differ on the open field and with time. It might be interesting to repeat the experiment in *Chapter* 4 on different soil types and under different environmental conditions on this account.

Notwithstanding the above considerations, this thesis's primary goal is to provide additional knowledge on low-input forage production systems' potential to mitigate GHG emissions. This thesis has revealed the impact of specific species and mixed-pastures on the GHG fluxes by showing the actual SOC stocks and N_2O-N emissions from low-input tropical and temperate systems. In addition, soil and botanical factors that might regulate C and N cycles under the prevailing environmental conditions have been identified. Accordingly, the results from this thesis could be helpful to the following stakeholders:

- Modellers: The data reported in this thesis can be used to fill gaps in data to enrich model outcomes when predicting C and N fluxes from tropical and temperate agroecological systems, respectively. These results provide accurate data that could be used with other data to develop proxies and models to ease data collection, particularly in developing countries, and reduce the high uncertainty associated with estimating GHG fluxes using process-based models or life cycle analyses.
- Policymakers: The results provide the basis for which strategies or policies could be formulated to address GHG mitigation at a broader scale. The N_2O EFs and range of SOC stocks reported in this thesis could provide the basis for estimating C and N_2O budgets for low-input systems when reporting to national, regional or international GHG accounting bodies. The challenges reported in this thesis might also inform policymakers to provide the needed infrastructure to foster agroecological research, particularly in low-income countries.

- Plant breeders can begin to breed forage species with a higher ability to allocate resources belowground, produce much more exudates, or have much higher N uptake efficiency. These traits might enhance pastures' overall capacity to mitigate GHG emissions and sequester much more C belowground.
- In the meantime, seed companies, farmer advisers/extension officers, ecologists and farmers could promote/adopt forage species/mixed pastures projected in this thesis as beneficial to enhance the agroecological environment.

Appendices

Appendix A

Table A1: Observed plant species according to land-use systems at the study site		
Cut-use fodder grasses		
Andropogon gabonensis	*Cenchrus setigerus*	*Eragrostis curvula*
Andropogon gayanus	*Chloris gayana*	*Eragrostis turgida*
Andropogon tectorum	*Chrysopogon aciculatus*	*Heteropogon contortus*
Brachiaria brizantha	*Cymbopogon giganteus*	*Hydropogon canaliculata*
Brachiaria mutica	*Cynodon dactylon*	*Hyperthelia dissoluta*
Brachiaria ruziziensis	*Cynodon plectostachyus*	*Panicum maximum (*Guinea fine*)*
Buchloe dactyloides	*Digitaria decumbens*	*Panicum maximum (*ILCA*)*
Cenchrus ciliaris	*Digitaria swanzilandensis*	*Panicum maximum (*local*)*
Cenchrus setigerus	*Digitaria valida*	*Panicum maximum (*South Africa*)*
Paspalum conjugatum	*Pennisetum ruppelii*	*Urochloa pullulans*
Paspalum dilatatum	*Seteria sp.*	*Vetiveria fulvivanus*
Paspalum notatum	*Seteria sphacelata*	*Vetiveria zizanioides*
Paspalum virgatum	*Sporobolus pyramidalis*	*Stenotaphrum dimidiatum*
Pennisetum purpureum	*Urochloa bolbodes*	*Tripsacum laxum*
Cut-use fodder herbs		
Calopogonium mucunoides	*Clitoria ternatea*	*Macroptilium atropurpureum (*Siratro*)*
Centrosema pubescens	*Desmodium distortum*	*Macroptilium lathyroides*
*Centrosema pubescens (*ILCA*)*	*Lablab purpureus*	*Mucuna pruriens*
*Mucuna pruriens (*Bunso	*Sesbania grandiflora*	*Stylosanthes hamata*
Pueraria phaseoloides	*Stylosanthes guianensis*	
Fodder trees and shrubs		
Baphia nitida	*Desmanthus virgatus*	*Leacaena lecocephala* (Peru*)*
Cajanus cajan	*Gliricidia sepium*	*Leucaena leococephla (*Langerham*)*
Cratylia argentea	*Leucaena leococephla (*local*)*	*Moringa oleifera*
Pithecelobium dulce	*Spondias mombin*	
Native grassland		
Acacia spp.	*Azadirachta indica*	*Cyperus rotundata*
Panicum decumbens	*Talinum triangulare*	*Elaeis guineensis*
Panicum maximum		
Seeded grazing fields		
Brachiaria brizantha	*Brachiaria ruziziensis*	*Digitaria decumbens*
Food crop fields		
Manihot esculenta	*Zea mays*	*Vigna unguiculata*
Arachis hypogaea		
NB: Names in parenthesis indicate cultivar name		

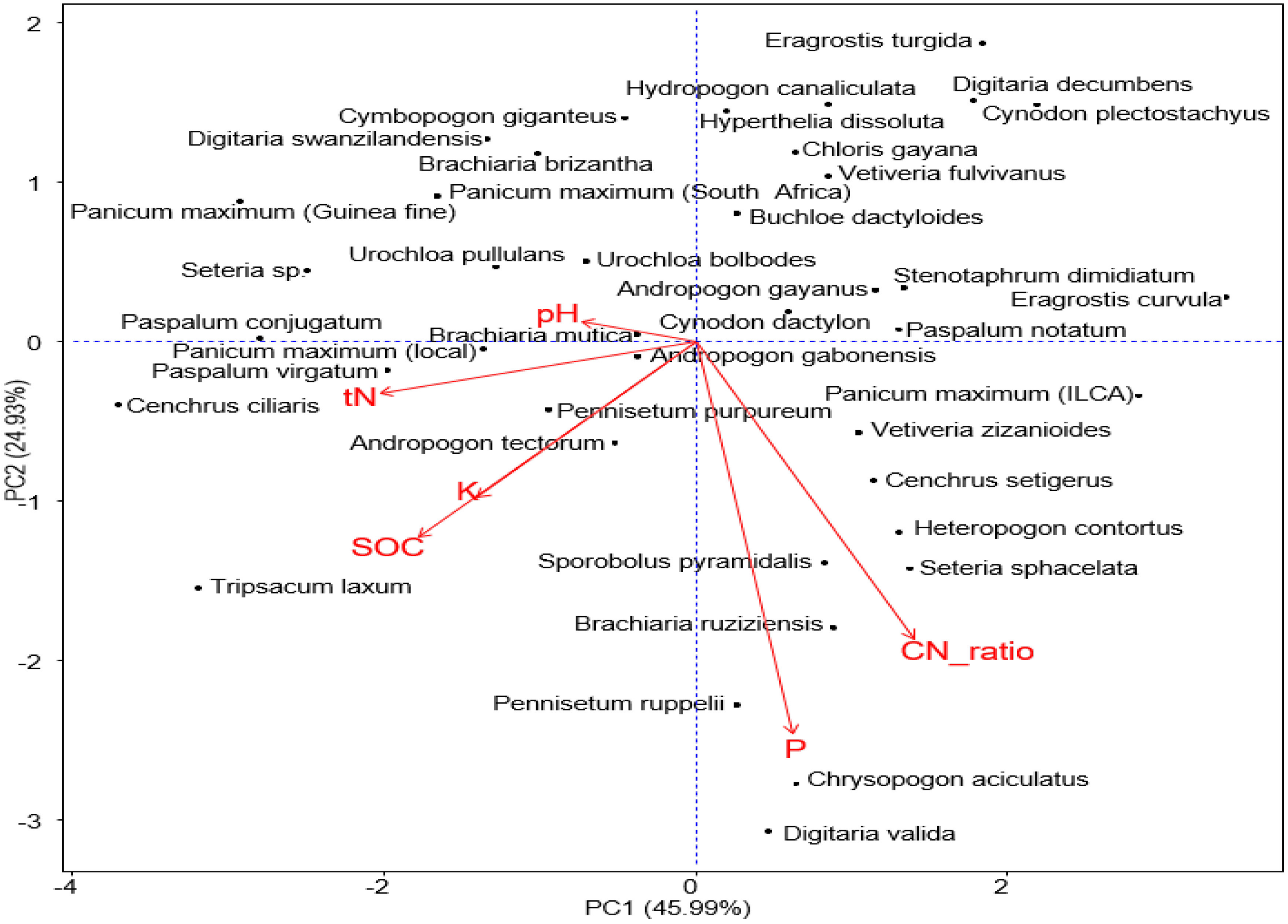


Figure A1. A plot of the first two PC loading vectors, describing the relationship among the variables (total nitrogen (tN), available P, exchangeable K, soil pH, C/N ratio) in the monocots, and plots of the first two PC score vectors, describing the classification of each tropical forage species within the PC loading vectors. The relationships among variables were determined by each variable's position in the loading plot. Accordingly, close variables have high correlations, variables on the opposite side of origin are negatively correlated, and those on the same side are positively correlated. Moreover, the magnitude of importance of each variable is determined by the distance from the origin.

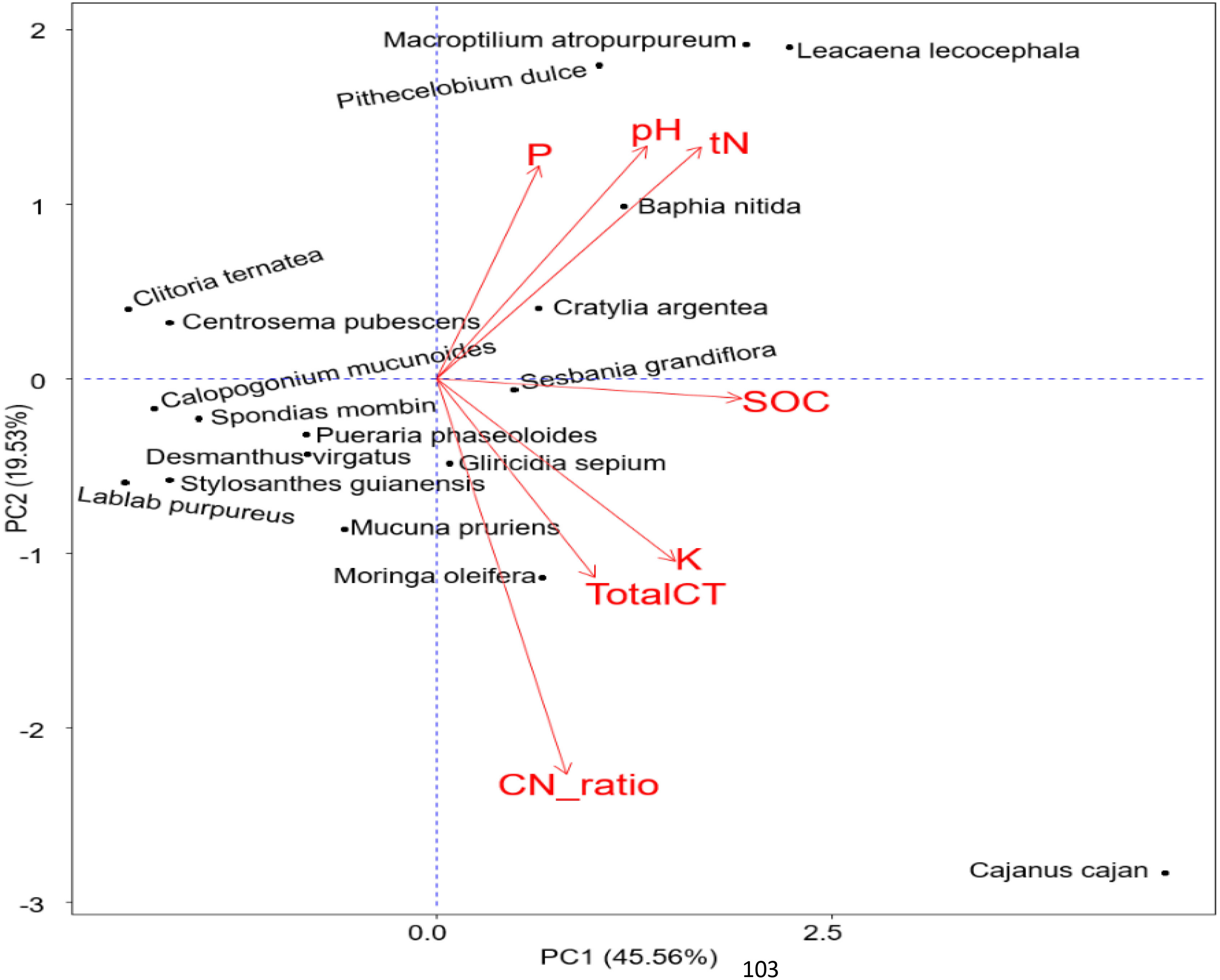


Figure A2. A plot of the first two PC loading vectors, describing the relationship among the variables (total nitrogen (tN), available P, exchangeable K, soil pH, C/N ratio, total condensed tannins (CT)) in the dicots, and plots of the first two PC score vectors, describing the classification of each tropical forage species within the PC loading vectors. The relationships among variables were determined by each variable's position in the loading plot. Accordingly, close variables have high correlations, variables on the opposite side of origin are negatively correlated, and those on the same side are positively correlated. Moreover, the magnitude of importance of each variable is determined by the distance from the origin.

Appendix B

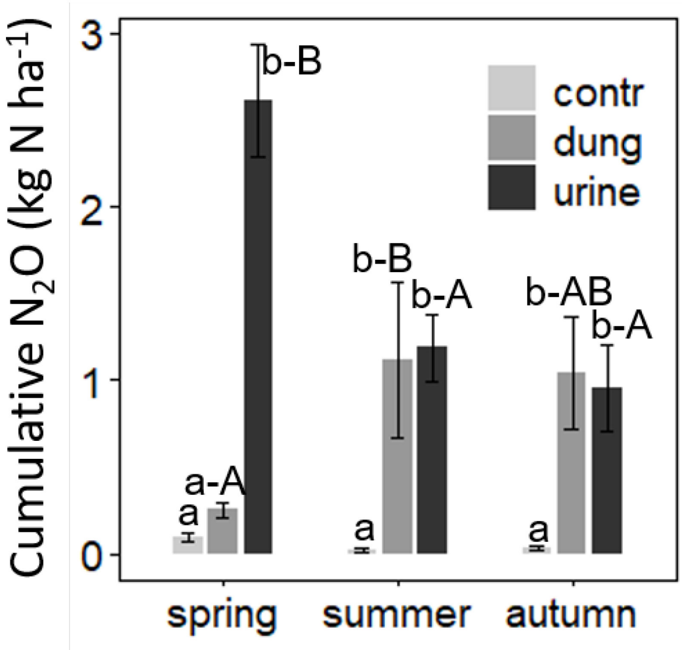


Figure B1. Graph showing season x treatment interaction effect (*P*<0.01) on cumulative N_2O emission irrigated binary-sward grasslands (Experiment 3). Means with different letters are significantly different [*P*<0.05; ^{abc} for the seasonal effect, ^{ABC} for treatment effect].

Table B1. Physical and chemical properties of the soil at the experimental site (30 cm soil depth).

Properties	
Soil texture	Sandy loam
Sand	61.1
Silt	26.2
Clay	12.7
Bulk density (g m^{-3})	1.56
Organic C (%)	2.2
pH	6.4
Total C (%)	1.29
Total N (%)	0.12
CN ratio	10.9
CEC (cmol kg^{-1})	17.2
Field capacity (%)	37.5

Table B2. Analyses of variance of the measured parameters showing main effects of pasture diversity (G), excreta type (T), and season (S) of excreta deposition on measured parameters.

Parameters	F-/P-Value						
	(G)rassland (2,52)	(T)reatment (2,52)	(S)eason (2,52)	G × T (4,52)	G × S (4,52)	T × S (4,52)	G × T × S (8,52)
	Experiment 2—Non-irrigated simulated grazing						
Grass (%)	94.4 ***	52.3 ***	2.45 ^{ns}	1.47 ^{ns}	2.84 *	1.88 ^{ns}	0.44 ^{ns}
Legume (%)	94.7 ***	62.7 ***	4.57 *	1.25 ^{ns}	5.36 **	0.66 ^{ns}	0.38 ^{ns}
Herb (%)	143.4 ***	2.72 ^{ns}	0.62 ^{ns}	3.66 *	0.62 ^{ns}	0.97 ^{ns}	0.97 ^{ns}
Unsown (%)	16.3 ***	3.76 *	2.85 ^{ns}	3.65 *	0.42 ^{ns}	0.24 ^{ns}	0.36 ^{ns}
DM yield (Mg ha^{-1})	44.0 ***	1.35 ^{ns}	39.3 ***	2.97 *	0.76 ^{ns}	15.2 ***	0.87 ^{ns}
N (g N kg DM^{-1})	9.4 ***	67.4 ***	291.0 ***	4.39 **	1.54 ^{ns}	26.9 ***	0.53 ^{ns}
$^{\beta}$ BFN (kg ha^{-1})	87.7 ***	79.0 ***	29.8 ***	4.63 **	6.96 ***	13.0 ***	0.69 ^{ns}
N input (kg N ha^{-1})	87.7 ***	512.7 ***	42.7 ***	4.63 **	6.96 ***	16.9 ***	0.69 ^{ns}
N uptake (kg N ha^{-1})	22.5 ***	6.44 **	5.77 **	2.43 ^{ns}	0.94 ^{ns}	21.8 ***	1.85 ^{ns}
PRN (kg N ha^{-1})	10.1 ***	45.7 ***	46.5 ***	3.40 *	3.11 *	19.7 ***	2.46 *
NO_3 (mg kg^{-1} soil d^{-1})	0.44 ^{ns}	64.4 ***	22.9 ***	1.06 ^{ns}	0.26 ^{ns}	4.54 **	1.03 ^{ns}
NH_4 (mg kg^{-1} soil d^{-1})	0.90 ^{ns}	29.6 ***	2.70 ^{ns}	1.30 ^{ns}	2.19 ^{ns}	2.27 ^{ns}	0.72 ^{ns}
N_2O-$N_{excreta}$ (kg N ha^{-1})	0.28 ^{ns}	51.6 ***	24.0 ***	1.80 ^{ns}	1.05 ^{ns}	6.06 ***	0.90 ^{ns}
N_2O (kg N ha^{-1} 100 day^{-1})	0.40 ^{ns}	10.5 **	17.6 ***	1.35 ^{ns}	0.57 ^{ns}	5.05 **	0.56 ^{ns}
N_2O-N EF (%)	0.63 ^{ns} (2,34)	0.005 ^{ns} (1,34)	34.4 *** (2,34)	3.37 * (2,34)	1.40 ^{ns} (4,34)	2.44 ^{ns} (2,34)	1.63 ^{ns} (4,34)
g N_2O-N kg^{-1}N uptake	0.31 ^{ns}	99.3 ***	34.0 ***	1.73 ^{ns}	0.71 ^{ns}	11.1 ***	1.04 ^{ns}
N_2O-N_{grazed} (kg N ha^{-1})	0.19 ^{ns}						
	Experiment 3—Irrigated simulated grazing						
Parameters		(T)reatment (2,24)	(S)eason (2,24)			T × S (4,24)	
NO_3 (mg kg^{-1} soil day^{-1})		79.8 ***	75.3 ***			16.9 ***	
NH_4 (mg kg^{-1} soil day^{-1})		135.9 ***	3.22 ^{ns}			3.19 *	
N_2O-$N_{excreta}$ (kg N ha^{-1})		53.9 ***	0.44 ^{ns}			8.68 ***	
N_2O-N EF (%)		8.37 * (1,15)	2.76 ^{ns} (2,15)			12.5 *** (2,15)	

DM: dry matter; Level of significance, $^{ns} = p > 0.05$, * < 0.05, ** < 0.01, *** < 0.001; $^{\beta}$ Estimated parameter. BFN: Biologically fixed nitrogen; N_2O-$N_{excreta}$: Emission from excreta patches; N_2O-N_{grazed}: Emitted from grazed fields. Figures in parenthesis indicate degrees of freedom followed by the sample size.

Table B3. Bivariate relationships between N_2O-N emission factors and the measured/estimated variables

Parameter	Estimate	*Adj.* R^2	F	Pr > F	Estimate	*Adj.* R^2	F	Pr > F
	Non-Irrigated Simulated Grazing				Irrigated Simulated Grazing			
Soil temp.	0.05	0.15	5.52	*	0.02	−0.02	0.38	ns
Precipitation	−0.01	0.14	5.27	*	2.75×10^{-4}	−0.03	0.13	ns
WFPS	−0.002	0.002	1.04	ns	−0.03	0.02	1.82	ns
WFPS^2	−0.002	0.13	2.92	*				
N content	1.19	0.21	9.65	**	6.54	0.17	5.82	*
C content	−0.10	−0.06	0.07	ns	−0.23	0.26	9.18	**
C content^2	−0.10	0.30	7.68	**				
C:N ratio	−0.002	−0.05	0.17	ns				
C:N ratio^2	−0.03	0.54	19.8	***	−0.08	0.22	7.64	*
N load	0.002	0.42	23.7	***	0.01	0.22	7.38	*
NH_4 intensity	-7.94×10^{-5}	0.07	2.29	ns	0.003	0.83	53.4	***
NO_3 intensity	-8.45×10^{-5}	0.37	10.8	**	0.002	0.61	18.4	**
Grass	−0.02	0.45	14.9	**				
Clover	0.02	0.52	19.1	***				
Clover:grass	0.50	0.63	30.1	***				
Herbs	−0.003	−0.06	0.04	ns				
Herbs:clover	−0.28	0.12	3.25	ns				
Herbs:grass	0.50	0.01	1.24	ns				
Dry matter yield	-1.21×10^{-4}	−0.05	0.22	ns	4.37×10^{-4}	0.10	4.87	*
Harvested N	−0.01	−0.002	0.98	ns				
N input	0.0003	0.03	2.85	ns				
N uptake	-8.93×10^{-4}	0.40	12.6	**				
PRN	7.0×10^{-4}	0.34	28.7	***				

Level of significance, ns = $p > 0.05$, * < 0.05, ** < 0.01, *** < 0.001; WFPS: Water-filled pore space.

Table B4: Effect of sampling days on the relationships (R^2) between N_2O-N emission and environmental factors (soil temperature and water-filled pore space)

Days after excreta application	15	30	60	100
Soil temperature (°C)	0.08	-0.03	0.07	0.26^{***}
Soil temperature ^2 (°C)	0.38^{***}	0.32^{***}	0.49^{***}	0.43^{***}
Water-filled pore space (%)	0.18^{**}	0.19^{**}	0.03	-0.004
Water-filled pore space^2 (%)	0.16^{*}	0.18^{*}	0.002	-0.03

‘ ’ = P> 0.05, * <0.05, **<0.01, ***<0.001

Appendix C

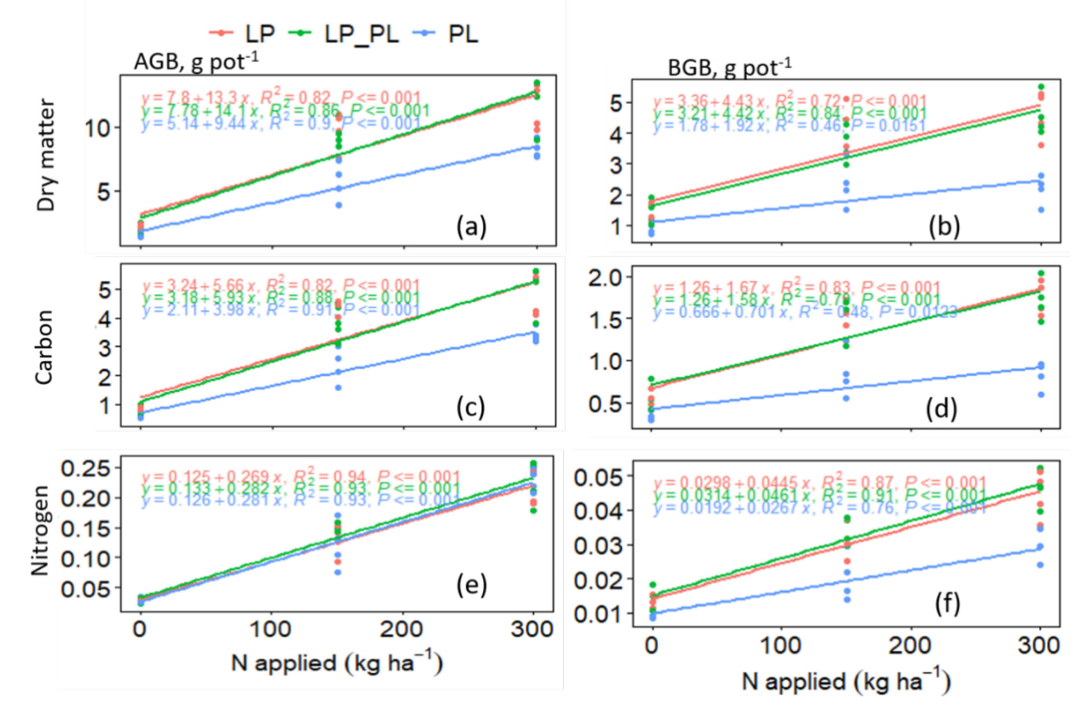


Figure C1. Graphs describing the linear relationships between N input and (a-b) dry matter (DM) yield, (c-d) biomass carbon yield and (e-f) biomass nitrogen yield. LP represents *Lolium perenne;* PL *represents Plantago lanceolate;* BGB – below ground biomass; AGB – aboveground biomass

Appendix D

The potential of dairy production in sub-Saharan Africa

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Vorträge und Posterbeiträge der 62. Jahrestagung der AGGF in Kiel. 223-227. ISBN 978-3-00-060516-1; 2018.

Introduction

There is a rapid increase in aggregate consumption of milk globally because of rising income levels, causing people to diversify from starch-based diets to diets containing growing amounts of dairy and meat (MUEHLHOFF *et al*., 2013). The demand for milk and meat is projected to double over the next two decades in Africa because of the faster population growth rate, particularly in the East and West (Figure A1). The ability of agriculture to meet this growing demand for animal food products hinges on sustainable agricultural practices, which must ensure food security, combat climate change and stimulate the development of new markets.

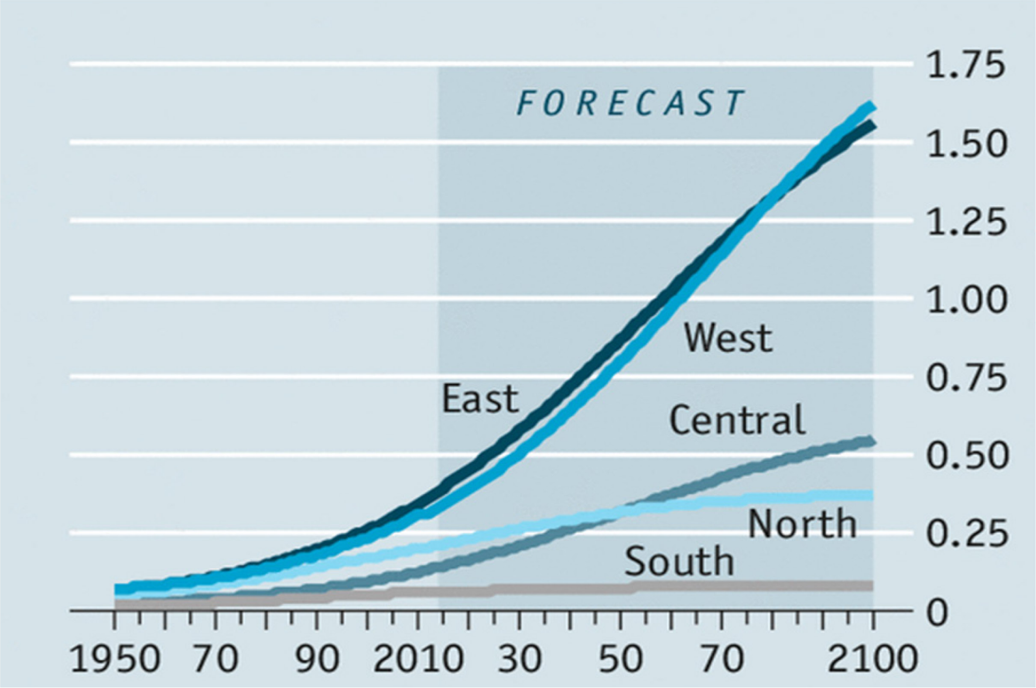

Figure. A1: Population trend of Africa (billion). Source: United Nations

Due to its vast available resources, Africa has the potential for economic development and to supply food resources to the rest of the world. However, consistent and committed actions are required by stakeholders towards improving the investment climate, close the huge infrastructure gap, focus more on innovation as the primary driver of productivity, growth and competitiveness and develop institutional and human capital (NDULU *et al*., 2007). Milk consumption per capita in Africa is the lowest in the world because of limited access and affordability (HILL, 2017). Although dairy products are traditionally not a significant part of many African diets, consumption has increased over the years. However, the continuous dependence on imported milk has discouraged local milk production and has led to increased cost of milk, putting milk consumption beyond the reach of the average African (ADEWUMI AND OLORUNNISOMO, 2008). This paper discusses the importance, status and ways to improve the dairy sector of sub-Saharan Africa (SSA).

The potential impact of dairy production in sub-Saharan Africa (SSA)

Increased availability and consumption of milk in Africa could accelerate the achievement of the sustainable development goals 1 (no poverty) and goal 3 (good health and well-being for people). Stunting, which reflects the cumulative effects of poor maternal nutrition, poor diet and infections during the first two years of life, is prevalent in Africa (MUEHLHOFF *et al*., 2013). Therefore, animal milk could be used as a supplement to breast milk and as an additional protein source to children, reducing child malnutrition, especially in children less than five years old. In addition, dairy consumption can translate into a substantive reduction in national healthcare costs. Dairy intake ensures a significant supply of various macro and micro nutrients that improve metabolic health and muscular-skeletal health and reduce dental caries, the incidence of cardio-vascular diseases, hypertension, and type-2 diabetes (HILL, 2017).

Increased availability and consumption of milk could positively impact the health of both infants and adults on the continent. More than 80 per cent of the milk produced in SSA comes from small-scale dairy producers. When well developed, dairy farming could improve food security and provide employment and income to millions of smallholder farm families. At larger scales of production, dairying could be an essential source of employment in marketing, processing and retailing, providing partial or total income for players in the dairy chain. In Kenya, for instance, every 1000L of milk produced generated full-time employments for 77 people in milk production and 3 - 20 jobs in processing and marketing and provided an average income 1.4 times higher than the Kenyan per capita GDP (WORLD BANK, 2003). In addition, the dairy sector generates year-round income, ensures higher labour productivity and promotes women empowerment (HILL, 2017). Undoubtedly, the dairy industry, when developed, could make a tremendous social impact on poverty alleviation, agricultural growth, combat malnutrition and increase the income levels of small-holder farm families in sub-Sahara Africa. It is often said that the socio-economic benefits of dairying are attained at an ecological cost. However, it is worth noting that over 77% of the feed for dairy animals in SSA is human–inedible pastures and straws that grow in rangelands. Moreover, a higher proportion (52 - 67%) of farm-based greenhouse gas emissions from dairy farms is methane, a relatively shorter-lived gas compared to CO_2 (5-10%) (FAO, 2010; HILL 2017). Invariably, several examples across the globe have demonstrated that improvements in breeding, feeding and management systems could increase milk production with reduced animal numbers, improved biological efficiency and, consequently, reduced environmental footprint (HILL, 2017).

Status and trends of milk production in Africa

The majority of the world's low-income tropical livestock producers are in Sub-Saharan Africa and South Asia, where dairy is produced in mixed crop-livestock systems, ranging from low-input, extensive grazing to more specialized intensive enterprises (MCDERMOTT *et al.*, 2010). Intensive systems are common in Sudan, Egypt, Kenya, South Africa, and Algeria, the top milk-producing countries on the continent regarding milk volume, producing about 52% of total African milk (SHITTU *et al.,* 2008). Apart from southern Africa, where high outputs have been reported, the rest of Africa is struggling to meet the milk demand of their respective countries. Much of the milk produced from the extensive system is for home consumption or sale at local markets, with only 5% of milk produced sold through commercial markets (USAID, 2013).

Nevertheless, milk production on the continent has doubled from 1996, with significant growth in North Africa, Kenya, and South Africa, with the other countries in Sub-Sahara Africa experiencing high relative growth but extremely low (PM FOOD AND DAIRY CONSULTING, 2014). Even though local production increased by 16.8% between 2005 and 2017 (1.5% per annum) (Figure A2a), undoubtedly, due to the increased number of milk cows by 27% (2.5% per annum), demand far outweighed supply. Meanwhile, milk output for 2018 has been estimated at 45.7 million tonnes, almost unchanged from 2016, with higher outputs in South Africa, Algeria and Tunisia that were primarily offset by lower production in Sudan, Ethiopia, Somalia and Tanzania (FAO, 2018). SHITTU *et al.* (2008) reported an average milk yield of 461kg ECM per cow, which was only one-fifth of the world average yield. An analysis of milk yield data from 2006 – 2016 showed a decrease of 8.1% (an annual decreasing rate of 0.74%) for Africa, whereas Europe and the world saw an increase of 2% and 11%, respectively, with a decreased milk cow population of 6% in the case of Europe (Figure A2c).

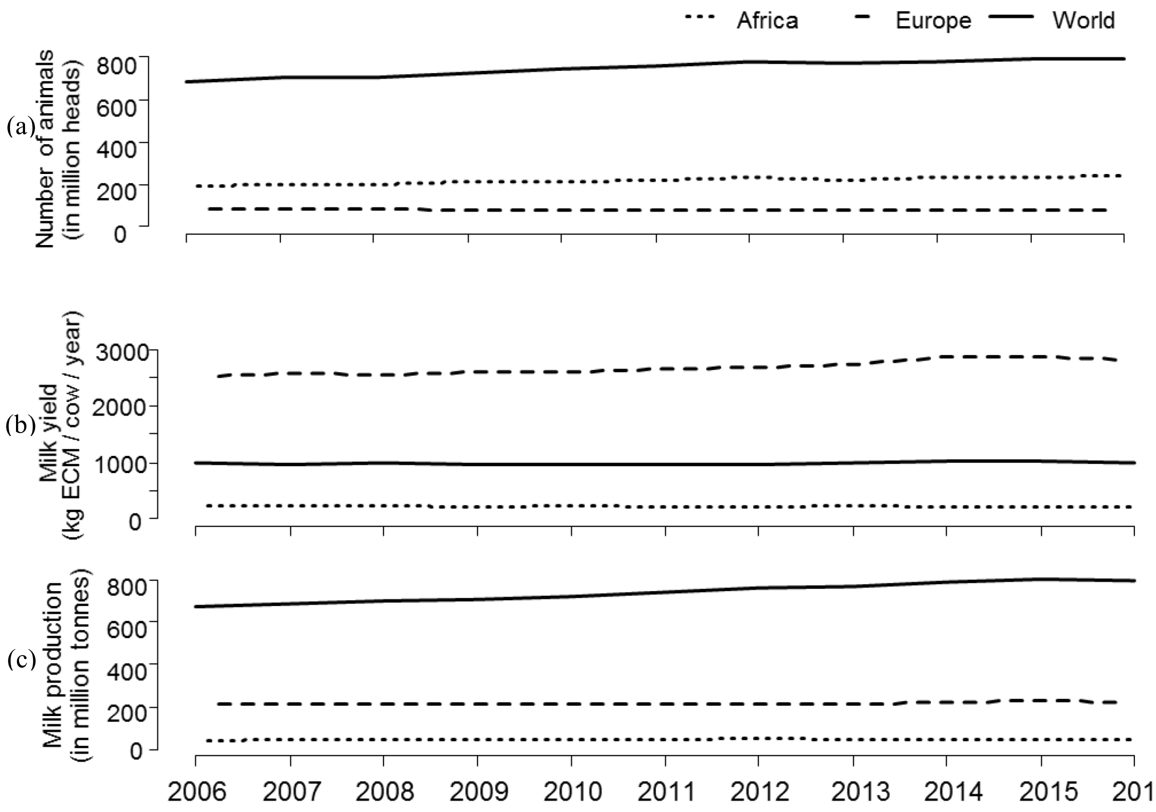

Figure A2: Changes in milk production (a), milk cow population (b) and milk yield in Africa compared with Europe and the world (2006 – 2016). Source of data: FAOSTATS (2018)

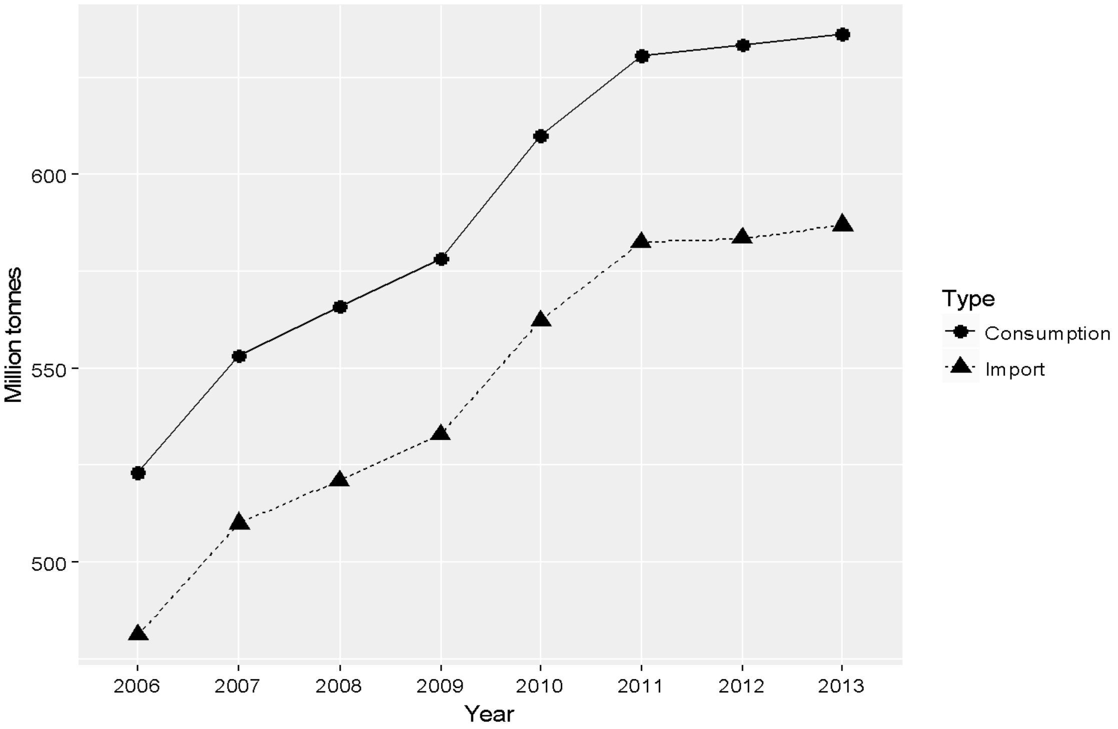

Figure A3: Milk consumption and imports trends in Africa Source of data: FAOSTATS

The decreasing trend in milk yield in Africa could be attributed to the negative impact of climate change on feed resources and animal performance (HIDOSA AND GUYO, 2017) since the majority of dairying in Africa is weather-dependent and probably, due to the deterioration of genes responsible for milk yield. Furthermore, the low milk production by SSA has led to huge imports of milk and milk products by many countries to augment local supply (Figure A3). With this development being exacerbated by continued climate change, measures are required to increase milk production drastically in SSA to meet the increasing demand.

Improving dairy production in SSA

It is suggested that the nature of the yield gaps in the dairy sector of Africa provides opportunities to increase production past the currently attainable yields despite the constraints being faced by the dairy sector (MAYBERRY *et al*., 2017). However, the right policies, marketing systems and technical support are needed to stimulate development in the dairy sector of SSA (ILRI, 2003). One of the major setbacks to development in SSA's dairy sector is a large number of local breeds with low milk-yielding capacity. Milk productivity of these local breeds has been below 200 litres per cow per year against 12,500 litres in some developed countries (DAVIS JR, 2018), although differences in production parameters exist. A study in one sub-Saharan country showed that almost all cattle were local breeds, with crossbred and exotic breeds such as Holstein-Friesian and Jerseys comprising only 1.3% of the national cattle herd (USAID, 2013). Replacing local livestock breeds with crossbred animals or exotic breeds is a plausible strategy to increase yield per cow. The selection of such productive breeds must be based on both economic and environmental considerations to ensure success.

Another challenge is the dominance of traditional systems for milk production, which account for over 90% of dairy production in Sub-Saharan Africa (OLALOKU AND DEBRE, 1992). These smallholder dairy production systems vary significantly according to the location, agro-ecological zone and socio-economic conditions (GIZAW *et al*. 2016). More than 80 per cent of farms in SSA are less than two hectares in size (LOWDER *et al.,* 2016). The small size of farms and their low level of creditworthiness create difficulty for producers to access credit. It is suggested that smallholder farmers pool resources together through cooperative systems to ensure economies of scale and consequently enhancing efficiency, productivity and market access. Banks could also assist in creating platforms to provide credit to dairy farmers and potential actors in the dairy value chain.

The major limitation to ruminant production in many tropical regions of Africa is poor nutrition. The productivity of animals is restricted by the low nitrogen and high fibre content of native grasses and crop residues which form the basis of the diets in these regions. The problem becomes aggravated during the dry season when pastures, cereal residues and maize stover limit nutritional quality. Therefore, interventions such as improving livestock nutrition are one effective strategy to improve milk yield and production. Communal grazing lands could be improved by reseeding natural pastures with perennial herbaceous legumes to increase nitrogen supply to soils and livestock and ensure soil carbon sequestration. In more intensive production scenarios, nutrition could be improved by increasing the amount and quality of supplements offered to livestock and increasing the nutritive value of crop residues by growing improved cultivars with higher metabolisable energy content (ANANDAN *et al.,* 2013).

Conclusion

The dairy industry has the propensity to create wealth for SSA, and governments of African countries must play a lead role in the transformation agenda. However, investment in scientific research has been very low in sub-Saharan Africa, making it challenging to develop home-grown technologies and policies to change the dynamics of dairying on the continent. With the continent already experiencing the adverse impact of climate change, it is vital to tackle the emerging issues of reducing feed quality and quantity, diseases, and environmental stress, which adversely influence milk production. African governments must be committed to ensuring the extension of transportation infrastructure to dairy production areas for quick transport of milk to processing facilities, ensuring cheap and reliable energy supply to processing plants, subsidising transportation costs, and stabilising fuel prices. Governments should ensure that farmers have access to improved dairy breeds and pest and disease management strategies. Regulations and policies are needed to safeguard grasslands which constitute a significant resource for the dairy industry. Proper grazing management systems must be adopted to increase yields, ensure yield stability and enhance ecosystem services. The current wave of population growth, land shortage, and increasing interest in production and consumption are expected to stimulate market-oriented dairy systems' emergence to ensure that sub-Saharan Africa derives the full benefits of dairy farming.

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EDUCATION AND TRAINING

01/10/2017 – 07/07/2021 – Department of Grassland and Forage Production/Organic Farming, Kiel, Germany
DOCTOR OF AGRICULTURE – Christian-Albrecht University of Kiel
Thesis: Diverse forage production systems and their impact on greenhouse gas mitigation

10/09/2020 – 10/09/2020
GPW YOUNG CROP SCIENCE ONLINE SCIENTIFIC WRITING WORKSHOP – Geschäftsführer der Gesellschaft für Pflanzenbauwissenschaften e.V.

11/02/2020 – 13/02/2020
SCIENTIFIC WRITING COURSE – Grünland und Futterbau/Ökologischer Landbau, Christian-Albrechts-Universität Kiel

18/04/2016 – 22/04/2016 – Dodowa, Ghana
CERTIFICATE OF ATTENDANCE, SCIENCE WRITING SKILLS – Council for Scientific and Industrial Reseach, Ghana and African Women in Agricultural Research

14/06/2015 – 18/06/2015 – Dodowa, Ghana
CERTIFICATE OF COMPLETION, MENTORING – Council for Scientific and Industrial Research (Ghana) and African Women in Agricultural Research

12/01/2015 – 16/01/2015 – Dakar, Senegal
CERTIFICATE OF ATTENDANCE, LIVESTOCK AND CLIMATE CHANGE – EU Animal Change Project

22/09/2014 – 03/10/2014 – Pretoria, South Africa
CERTIFICATE OF ATTENDANCE, GREENHOUSE GAS MEASUREMENT FROM LIVESTOCK SYSTEMS – University of Pretoria and Global Research Alliance

14/10/2013 – 18/10/2013 – Accra, Ghana
CERTIFICATE OF PARTICIPATION (Proposal writing, Scientific writing, Project management and intellectual Property) – Council for Scientific and Industrial Research, Ghana

11/03/2013 – 15/03/2013 – Kumasi, Ghana
CERTIFICATE OF ATTENDANCE, CLIMATE CHANGE PROCESSES, MITIGATION AND ADAPTATION – Aarhus University (Denmark) and Kwame Nkrumah University of Science and Technology (Ghana)

01/08/2006 – 31/07/2008 – Kumasi, Ghana
MASTER OF SCIENCE, ANIMAL NUTRITION, FEBRUARY 2010 – Kwame Nkrumah University of Science and Technology
Thesis: Productive performance and carcss characteristics of captive grasscutters (*Thryonomys swinderianus*)

01/09/1999 – 31/07/2003 – Cape Coast, Ghana
BACHELOR OF EDUCATION, AGRICULTURE (FIRST CLASS HONOURS), AUGUST 2003 – University of Cape Coast (University College of Education, Winneba)
Thesis: Pest Incidence, Mortality, Aestivation, Feed Intake and Growth in West African Giant Snails

01/08/1994 – 30/06/1997 – Hohoe, Ghana
TEACHER'S CERTIFICATE "A", JUNE 1997 – University of Cape Coast (St. Francis Training College)

01/09/1989 – 30/06/1994 – Kpando, Ghana
GENERAL CERTIFICATE OF EDUCATION (ORDINARY LEVEL), JUNE 1994 – Kpando Secondary School

01/09/1986 – 30/06/1989 – Kpando, Ghana
BASIC EDUCATION1 – Kpando Kpodzi Evangelical Presbitarian Middle School

01/09/1984 – 30/06/1986 – Atiwlame (Afram Plains), Ghana
BASIC EDUCATION 2 – Atiwlame Local Authority Primary School

01/09/1980 – 30/06/1984 – Nkonya Ahenkro, Ghana
BASIC EDUCATION 3 – Nkonya Ahenkro Roman Catholic Primary School

WORK EXPERIENCE

02/10/2012 – 30/06/2017
RESEARCH SCIENTIST – COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH - ANIMAL RESEARCH INSTITUTE

01/09/2003 – 30/09/2012
SECONDARY EDUCATION TEACHING PROFESSIONAL – GHANA EDUCATION SERVICE

01/07/2000 – 30/09/2000
INDUSTRIAL ATTACHMENT – EVANGELICAL PRESBYTERIAN CHURCH HO FARMS

01/09/1997 – 30/07/1999
SECONDARY EDUCATION TEACHING PROFESSIONAL – GHANA EDUCATION SERVICE (ALOKPATSA L/A JUNIOR SECONDARY SCHOOL)

PUBLICATIONS

Nitrous oxide emission from grazing is low across a gradient of plant functional diversity and soil conditions.
Nyameasem JK, Malisch CS, Loges R, Taube F, Kluß C, Vogeler I & Reinsch T. Atmosphere, 12(2):223. https://doi.org/10.3390/atmos12020223 – 2021

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Reproductive traits of the indigenous Guinea fowl under tropical humid conditions; the effect of egg size.
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http://www.lrrd.org/lrrd32/4/gadua32055.html – 2020

Protein and energy requirements for indigenous guinea keets (*Numida meleagris*) in southern Ghana
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Ghana Journal of Agricultural Science, 52. ttps://www.ajol.info/index.php/gjas/article/view/179721 – 2018

Effects of protein content on feed intake and performance of grasscutters fed diets containing forage meal
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Wallace PA, Nyameasem JK, Aboagye GA, Affedzie-Obresi S, Nkegbe K, Murray F, Botchway V, Karbo N, Leschen W,
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Impact of black soldier fly larval meal on growth performance, apparent digestibility, haematological and blood chemistry indices of guinea fowl starter keets under tropical conditions
Wallace PA, Nyameasem JK, Adu-Aboagye GA, Affedzie-Obresi S, Nkegbe EK, Karbo N, Murray F, Leschen W & Maquart P-O
Tropical Animal Health & Production, 49, 1163–1169. https://doi.org/10.1007/s11250-017-1312-x – 2017

The grasscutter: an untapped resource of Africa's grasslands
Adu EK, Asafu-Adjaye A, Hagan BA & Nyameasem JK
Livestock Research for Rural Development, 29, 47.
http://www.lrrd.org/lrrd29/3/jnya29047.html – 2017

Nitrogen partitioning and growth in grasscutters fed freshly cut *Panicum maximum* alone or supplemented with *Leucaena lecocephala* treated with 0, 1 or 2 per cent saline solution
Nyameasem JK, Amoah KO, Wallace PA & Adu EK
Livestock Research for Rural Development, 29, 24.
http://www.lrrd.org/lrrd29/2/nyam29024.htm – 2017

The effect of male proximity on vagina patency, oestrus cycle length and feed intake of female grasscutters.
Nyameasem JK, Adu EK, Amoah KO & Hagan BA
Tropical Animal Health & Production, 48, 445–449. https://doi.org/10.1007/s11250-015-0972-7 – 2016

Predicting live weight of grasscutters using their linear body measurements
Hagan BA, Nyameasem JK, Asafu-Adjaye A & Daffour-Oduro KA
Livestock Research for Rural Development, 28, 143.
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Effects of non-genetic factors on the birth weight, litter size and pre-weaning survivability of West African Dwarf goats in the Accra Plains
Hagan BA, Nyameasem JK, Asafu-Adjaye A & Duncan JL
Livestock Research for Rural Development, 26, 13.
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Effect of formulated diets on growth and reproductive performance of the West African giant snail (*Achatina achatina*)
Nyameasem JK & Borketey-La EB
ARPN Journal of Agricultural and Biological Science, 9, 1. http://www.arpnjournals.com/jabs/research_papers/rp_2014/jabs_0114_626.pdf – 2014

Productive performance and Carcass Characteristics of Captive *Thryonomys swinderianus* fed Concentrate diets containing varying levels of *Panicum maximum*
Karikari PK & Nyameasem JK
World Applied Sciences Journal, 6 (4): 557-563. http://www.idosi.org/wasj/wasj6(4)/18.pdf – 2009

CURRENT PROJECT
Nitrous oxide emission from forage plantain and perennial ryegrass swards is affected by resource allocation dynamics belowground
John K. Nyameasem, Enis Ben Halima, Carsten S. Malisch, Bahar S. Razavi, Friedhelm Taube, Thorsten Reinsch

CONFERENCES AND SEMINARS

19/10/2020 – 21/10/2020 – Helsinki, Finland
28th EGF General Meeting 2020
“Meeting the future demands for grassland production”

18/09/2019 – 20/09/2019 – University of Kassel, Germany
Tropentag 2019
“Filling gaps and removing traps for sustainable resources management”

29/08/2019 – 31/08/2019 – Raitenhaslach, Germany
63. Jahrestagung der AGGF 2019 in Raitenhaslach: Grünland 2050
"Grünland 2050"

27/09/2018 – 28/09/2018 – Universität Hamburg, Germany
Workshop of the working group ‘Soil Gases’ of the German Soil Science Society

"How can soil process research on various scales contribute to global greenhouse gas modeling?"

10/09/2018 – 13/09/2018 – Berlin, Germany
International Conference on Agricultural Greenhouse Gas Emissions and Food Security
"Connecting research to policy and practice"

30/08/2018 – 01/09/2018 – Kiel, Germany
62. Jahrestagung der AGGF in Kiel
"Leistungen von Gras und Klee-Gras auf Acker und Grünland"

LEADERSHIP/ORGANISATIONAL SKILLS

- Leadership skills gained during my work as head of department with Ghana Education Service; and from project team management
- Good organizational skills gained during my activities as secretary to academic board and coordination of projects

COMMUNICATION AND INTERPERSONAL SKILLS

- Communication skills gained through my experience from teaching students and training of farmers.
- Presentation skills gained during academic and professional training

JOB-RELATED SKILLS

- Scientific writing skills (I have led a number of manuscript write-ups)
- Mentoring skills gained through training and experience (I have voluntarily mentored many students from
  University of Education, Winneba when I was teaching, and also I mentor university students on internships and
  those doing scientific research)
- Presentation and report writing skills gained from academic works and research activities
- Life cycle analyses, modeling using APSIM

HOBBIES

- Reading
- Listening to music & dancing
- Football watching/playing
- Sightseeing

NETWORKS AND MEMBERSHIPS

- Ghana Science Association
- Ghana Society of Animal Production
- Global Research Alliance - Integrated Research Group

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