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Legumes and livestock in no-till crop rotations: Effects on nitrous oxide emissions, carbon sequestration, yield, and wheat protein content

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HIGHLIGHTS

- Crop rotation strategies have legacy effects on production and climate impact.
- Rotations without livestock: low N_2O emissions, but low yield, high fertiliser need.
- Dropping wheat protein of cash crop-only rotations over 20 years were unsustainable.
- Crop rotations with legumes/livestock had better wheat yields and grain quality.
- Emission hotspots are off-season, making reduction hard.

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ABSTRACT

Context: Crop rotation is seen as a 'Climate-Smart Agriculture' practice, but there are knowledge gaps around their climate impacts. This is the first direct measurement of nitrous oxide emissions (N_2O) from cropland soils in South Africa.

Objective: Assess the production performance, soil greenhouse gas emissions, and soil carbon sequestration of different crop rotations.

Methods: Continuous measurement over one year of direct soil N_2O and methane fluxes and analysis of 20 years of historical data on soil carbon sequestration, yields, fertiliser applications, and wheat (*Triticum aestivum*) protein content.

Results and conclusions: Rotations that contained legumes and livestock produced higher wheat (3.5–3.6 vs 3.1 t ha^{-1} $year^{-1}$) and canola yields (1.5–1.8 vs 1.3 t ha^{-1} $year^{-1}$) with superior wheat protein contents, while the cash crop only system's protein content decreased by 0.085 absolute % points annually (compared to 0.01–0.05 %). The results suggest a strong crop rotation legacy effect on the accumulation and availability of nitrogen in the soil profile, for both crop growth and N_2O production, where systems which integrated legumes and livestock vs. cash crops only had 0.31–0.42 vs. 0.14 kg N_2O-N ha^{-1} $year^{-1}$. All systems showed a significant increase of soil organic carbon of 0.24–0.30 Mg C ha^{-1} $year^{-1}$ over the 20-year period.

Significance: Legumes and livestock incorporation in crop rotations interact with nitrogen management. Most N_2O emissions occurred after precipitation in the otherwise dry summer, making reduction difficult as minimal management activities occur over this fallow period. A significant challenge in designing N_2O mitigation strategies is the lack of existing N_2O flux datasets needed to develop specific, regional emission factors.

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1. Introduction

Mitigation of greenhouse (GHG) emissions in agricultural systems are critical to limit climate change. Direct GHG emissions from agricultural soils account for 11 % of all GHGs produced by the 'Agriculture, Forestry, and Other Land Use' sector (IPCC, 2022). In the Western Cape province of South Africa, dryland crop and crop-pasture systems dominate the landscape. Similar systems exist in Australia, Mediterranean Europe, and South America (Kassam et al., 2012). Despite this region being a significant producer of small grains and governmental prioritisation of 'Climate-Smart Agriculture', there is a knowledge gap regarding soil GHG emissions.

Soil emissions vary spatially and temporally as they are produced through several biochemical processes facilitated by soil biota and fluctuating environmental conditions. The main soil GHGs are carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4) (IPCC, 2022). Arid soils, such as in the Western Cape, are generally weak CH_4 sinks, oxidising CH_4 before it can leave the soil profile (Shumba et al., 2023). Cropland soils, however, are the primary source of N_2O emissions (Biernat et al., 2020), and mitigating N_2O release is the most strategic action to reduce soil GHG emissions. With increasing soil temperatures, water-filled pore space, and soil nitrogen (N) inputs (mineral fertilisers, crop residues, biological N fixation, or manure) N_2O fluxes increase (Liu et al., 2022; Ruser et al., 2006). Emissions are exacerbated when soil N is in excess of plant uptake, making N management in crop production a critical lever (Shcherbak et al., 2014; Smit et al., 2020).

In the initial conversion of natural areas into intensive agricultural land, a significant loss of soil organic C to the atmosphere typically occurs (Lal, 2001). By modifying management through tillage reduction, residue retention and organic inputs, and crop rotation (especially with pasture or high root biomass crops), soil C increases are potentially promoted (Paustian et al., 2016). The slow build-up of soil C from a relatively degraded soil results in a sink of CO_2, decreasing the atmospheric pool by increasing the terrestrial pool. This sink is temporary, as several decades following "best soil management", a soil organic C steady-state determined by C inputs, soil and environmental factors is reached, and increases in soil organic C plateau (Minasny et al., 2017). Over large areas of land, incremental increases in soil organic C can equate to large amounts of CO_2, making soil C sequestration is another 'climate-smart' focus.

The effect of a crop rotation system compounds over time, as patterns of biomass production, management practices, and inputs repeat. These factors manipulate the production or sequestration of GHGs (Barton et al., 2013; Liebig et al., 2020; Pravia et al., 2019), sometimes with a factor having opposite effects on different GHGs; for example, the addition of organic material could increase CO_2 sequestration while increasing N_2O emissions (Chen et al., 2021; Manalil et al., 2014).

Due to this complexity, estimated soil emissions are highly uncertain, which is exacerbated in data-scarce regions, such as the Western Cape, South Africa (Reinecke and Casey, 2017). The IPCC's Guidelines for National Greenhouse Gas Inventories tier 1 method is the norm for estimating soil N_2O emissions; countries use it to calculate their Nationally Determined Contributions as per the Paris Climate Agreement, unless regional data is available and higher tier methods are used (IPCC, 2006) and forms part of The Greenhouse Gas Protocol's widely used GHG corporate accounting standards (Kasperzak et al., 2023; WRI, WBCSD, and GHG Protocol, 2022). Having many regional datasets of direct N_2O emissions is required before more accurate higher tier methods (tier 2 and 3) can be used. Accurate quantification is essential in assessing a system's impact on climate change and comparing different production modes and interventions (Galloway et al., 2024). This experiment aims to directly quantify N_2O and CH_4 fluxes and long-term soil C sequestration to ascertain both the GHG status and to compare different crop rotation systems.

2. Materials and methods

2.1. Experimental site overview

The experimental site was situated on Langgewens Research Farm (−33.27665°; 18.70463°; 191 m elevation) near Moorreesburg, in the Western Cape province of South Africa. This region, known as the 'Swartland', is a vital dryland cropping area for small grains, typically wheat (*Triticum aestivum*), canola (*Brassica napus*), barley (*Hordeum vulgare*), and lupin (*Lupinus* spp.). Livestock production systems consist of Letelle sheep, a Merino-type dual-purpose breed, grazed on annual medic (*Medicago trancutula* and *M. polymorpha*) pastures during the growing season and grazed on dry crop and medic residues in the fallow period. The region has a Mediterranean climate. Average temperatures range between 12.2 °C and 24.2 °C. The average annual precipitation is 395 mm, distributed with 80 % occurring in the winter production season between April to September. See Supplementary Fig. A.1 for the 2021 – 2022 daily temperatures and distribution of precipitation on Langgewens Research Farm, Western Cape, South Africa. Soils comprise shallow, sandy loam soils derived from Malmesbury and Bokkeveld shales, which tend to drain laterally instead of vertically. The primary soil forms are Swartland and Glenrosa, with world reference base classifications: Stagnic Lixisol (abruptic, clayic) and Leptic Lixisol (abruptic, skeletic), respectively (Wiese et al., 2016). The average clay content was 11.2 % (range of 9–16 %), and the average pH (KCl) was 5.8 (range of 5.4–6.3). The soil conditions offered a rooting depth of 40–60 cm and varied across the trial, with texture, coarse fragment content, and landscape position.

2.2. Experimental layout

The site consisted of a long-term crop rotation trial (in its 25th production year) of three different four-year crop rotation systems. Field plots varied between half-hectare and two-hectare plots in a randomised block design. As plot sizes varied, all results were standardised to one hectare. Each phase of the four-year rotation system was represented yearly and replicated twice, resulting in eight experimental plots per rotation system. The rotations had varying levels of livestock and legume incorporation.

The three representative rotation systems chosen for monitoring in this study were: Wheat – wheat – wheat – canola (WWWC); Wheat – canola – wheat – cover crop mixture (WCWL); and Wheat – medic pasture – canola – medic pasture (WMCM). The cover crop phase consisted of a mixture of legumes, brassicas, and fodder cereals. Historically, lupins were planted from 2000 to 2015 and replaced with a cover crop mixture from 2016 to 2021. See Supplementary Table A.2 for composition seeding density and cultivars used.

All systems followed conservation agriculture principles of crop rotation, residue retention, and minimum soil disturbance. A zero-tillage double disc seeder (Piket Implements, Piketberg, South Africa) planted crops at a row spacing of 254 mm, except for annual medics, which regenerates from the seed bank. All crop residues were retained on WWWC and WCWL, with less than 40 % of the residues grazed in MWMC. The cover crop phase produced approximately 5000 kg ha^{-1} dry material and was intensively grazed by sheep twice throughout the season at a stocking rate of 140 large livestock units (LLU) ha^{-1}. The average grazing period on the annual medic pastures during the growing season (April/May – October/November) was 15 days at a stocking rate of 35 LLU ha^{-1}. The total annual medic pasture production was estimated at 4000 kg ha^{-1} based on prior research published at Langgewens Research Farm (Swanepoel and Tshuma, 2017). Over the fallow season, the livestock were rotated across all systems, staying for approximately one week at 35 LLU ha^{-1} to spread the crop residues. Supplementary Table A. 2 provides an overview of precise grazing events for different plots.

Each rotation system followed a unique fertilisation and pesticide program determined according to field plot needs. Measurement overlapped two cropping seasons, resulting in a unique program for each plot, in line with best practice recommendations for local farming. Detailed N fertilisation program and agrochemical lists are in Supplementary Tables A.3 and A.4, respectively. Nitrogen fertiliser rates are determined according to guidelines that consider the crop's production potential (i.e., rainfall), crop rotation system, and soil texture. Typical yields for this region is 2.5–4 t ha^{-1} $year^{-1}$ for wheat and 1–2 t ha^{-1} $year^{-1}$ for canola. In the WWWC system, the total N applied was 87 kg N ha^{-1} $year^{-1}$ for wheat and 80 kg N ha^{-1} $year^{-1}$ for canola. In WCWL, the cover crop phase included a N-fixing legume decreasing the fertiliser requirement for the wheat phase in the following year to 72 N ha^{-1} $year^{-1}$, the wheat phase following canola received 87 kg N ha^{-1} $year^{-1}$ and canola received 80 kg N ha^{-1} $year^{-1}$. The cover crop phase was given only 5 kg N ha^{-1} $year^{-1}$ at planting. In WMCM, the annual medic phases provided biologically fixed N for the system; wheat received 26 kg N ha^{-1} $year^{-1}$, and canola received 48 kg N ha^{-1} $year^{-1}$.

2.3. Determination of agronomic parameters

Aboveground biomass samples were taken 60- and 90 days following plant emergence and before harvest. For wheat, biomass was estimated using three 1 m strips and converting values into square meter units. Canola biomass was determined using the average biomass of ten plants and multiplied by a plant population count. Plant population counts were estimated 60 days after emergence by averaging 15 random 1 m row counts. Samples were oven-dried at 60 °C for 48 h before weight determination. Relative biomass was calculated by dividing the mass of each plot response by the maximum weight achieved for each sampling event. The harvest index was calculated by dividing yield by biomass before harvest. A rough estimate for the quantity of crop residues left behind after harvest was taken as the difference between yield and biomass before harvest.

The long-term trial records provided data on yield, wheat protein content, and N fertiliser inputs. As no soil nitrate values were recorded in the long-term trial, wheat protein values were used to give insight into plant available N. Differences in soil N are expected across rotations due to the long-term inputs of legume residues and animal excreta. Historical yield, protein and grading for the period 2000–2021 were retrieved from quality reports provided by the silo to which the grain was sold. Wheat protein content was determined with a near-infrared (NIR) grain analyser (model IM 9500, Perten Instruments, Waltham, USA). Nitrogen exported (kg N ha^{-1}), depicting the amount of N removed in the harvested grain, was calculated according to the following formula:

$$\textbf{Nitrogen exported}\ (\textbf{kg N ha}^{-1}) = \text{Protein}\ (\text{g g}^{-1}) \times \text{Yield}\ (\text{kg ha}^{-1}) \times \text{N content in crude protein} \tag{1}$$

where *N content in crude protein* is taken as a factor of 0.16

2.4. Carbon sequestration monitoring

Historical routine soil fertility test reports for the Langgewens trial were used to obtain soil organic C content from 2000 to 2021 (except for 2004, for which organic C content was not determined). For each field plot, two composite samples consisting of 40 random samples sampled to a depth of 15 cm were taken for each field over the summer fallow period. The Walkley-Black method (Nelson and Sommers, 1982) was used to determine soil organic C content. A soil particle size distribution was determined in the 2016 soil test using the hydrometer method (Gavlak et al., 2005), and bulk densities were determined in the 2021 soil sampling via the intact core method. Coarse fractions (particles >2 mm) were obtained in 2010 from a grid of soil profiles, which classified the topsoil coarse fraction into distinct volume classes of 10 %. The six proximate profiles were averaged to obtain a plot value. Organic C stocks (Mg ha^{-1}), were calculated with the equation used by the IPCC's Guidelines for National GHG Inventories and FAO's GSOC MRV protocol and was selected as it takes coarse fraction, a substantial component of this region's soils, into account. The following formula was used:

$$\text{Carbon stocks}(\text{Mg ha}^{-1}) = \text{Soil organic carbon\%} \times \text{Soil depth}\ (0.15\ \text{m}) \times \text{Bulk density}\ (\text{Mg m}^{-3}) \times (1 - \text{Coarse fraction}) \times 10^4 \tag{2}$$

2.5. Nitrous oxide and methane fluxes quantification

The closed manual static chamber method was used to determine N_2O and CH_4 fluxes (Hutchinson and Mosier, 1981). One chamber was placed per experimental plot ($n = 24$). Chambers were made from Polyvinyl chloride (PVC) pipe sections with a 50 cm diameter and comprised of 15 cm high collars, 50 cm high extensions and 35 cm high lids.

The ends of the soil collars were sharpened to a 45-degree angle for easy insertion into the soil. The lid pipe sections were each closed with a 3 mm thick PVC foam board and attached via PVC cement. The exterior of the lids was covered with reflective aluminium foil tape, and the extensions were spray-painted white to increase surface albedo and reduce internal heat build-up. Extensions were used to extend the total chamber to accommodate the growing plants. The following different chamber heights were achieved: 0.4 m (lid only), 0.9 m (lid and one extension), 1.4 m (lid with two extensions). All components (collar, extension, and lids) could be connected via canopy clips, and closed cell foam strips were glued between each join to ensure a seal. A 50 cm long, 4 mm coiled vent was inserted into the side of the lid to maintain a pressure equilibrium. On top of the lid, halfway between the centre and the edge, an 18 mm butyl rubber injection port was inserted into a 12 mm hole and taped closed. Temperature sensors were inserted into two chambers on opposite sides of the trials. They were inserted so the sensor hung into the chamber through a small hole sealed up with reusable putty adhesive while the display remained outside. Soil collars were inserted 10 cm deep into the ground one week before sampling to avoid experimental noise caused by soil disturbance during installation. The plots contained ridges and troughs; collars were placed halfway up the slope. Collars were situated at least ten meters inside the field away from wheel tracks to ensure representative soil conditions. Collars were placed to surround two crop rows. Throughout the sampling period, collar reinsertion (e.g., at crop planting) was done at least 48 h before the subsequent sampling took place.

Sampling commenced one month after planting for one year (3 June 2021–8 June 2022). Samples were taken weekly during the growing season (planting to harvest) and every second week in the fallow summer season (harvest to planting). Sampling was conducted between 9:00–11:30 AM each sampling day (with few exceptions) as this time is representative of the daily mean (Alves et al., 2012). Thirty millilitres air samples were drawn using a 60 ml syringe fitted with a 21G needle on a three-way stopcock and injected into 12 ml pre-evacuated Labco© exetainers. Samples were taken immediately following chamber closure at intervals of 0, 20, 40, and 60 min. Inspection of all equipment took place before any sampling. The 24 chambers were separated into three routes of eight chambers each, which were run in parallel. Climatic data used in flux analysis was logged on a weather station at the research farm. Samples were analysed by gas chromatography (SCION 456-GC, Bruker, Leiderdorp, The Netherlands) using a 63Ni electron-capture-detector and Helium as the carrier gas.

The following formula was used to calculate N_2O and CH_4 fluxes from the air samples:

$$Gas\,flux\left(\mu g m^{-3} h^{-1}\right) = k \times \left(\frac{273.15}{T+273.15}\right) \times \left(\frac{Chamber\,volume}{Soil\,area\,in\,chamber}\right) \times \left(\frac{\Delta c}{\Delta t}\right) \quad (3)$$

where k is a conversion unit for different gases, for N_2O: k = 1.25 μg N μl^{-1}, and for CH_4: k = 0.536 μg C μl^{-1}, T is Temperature inside the chamber in °C and $\frac{\Delta c}{\Delta t}$ represents the change in gas concentration in the chamber over time in $\mu l\, l^{-1}\, h^{-1}$, as determined by modelling gas samples on a linear regression.

The GWP100-AR6 metric was used to weight CH_4 and N_2O into carbon dioxide equivalents (CO_2-eq), where CH_4 (non-fossil) = 27.0 ± 11 and N_2O = 273 ± 130 CO_2-eq. It is preferable not to aggregate different GHGs via an emission metric, as GHGs differ in radiative forcing and lifespans, this can introduce ambiguity in climate impact estimations (IPCC, 2021). All values are reported per kg N_2O-N or CH_4, with CO_2-eq values only used to evaluate the systems on the relative contribution of different GHGs in the discussion section.

To compare field measures to a modelled estimate, the IPCC Guidelines for National Greenhouse Gas Inventories, Tier 1 method was used:

$$\text{Nitrous oxide emissions}\left(\text{ kg } N_2O - N \text{ ha}^{-1}\text{year}^{-1}\right) = \left(\left(F_{SN} + F_{ON} + F_{CR} + F_{SOM}\right) \times EF_1\right) + \left(F_{PRP} \times EF_{PRP}\right) \quad (4)$$

where F_{SN} is kg synthetic N fertiliser applied, F_{ON} is kg N organic inputs applied, F_{SOM} is kg N mineralised during loss of soil organic matter (assumed to be zero). F_{CR}, kg N in crop residues was calculated based on biomass sampling before harvest, yield, and default IPCC factors for below-ground biomass and N concentration. A 40 % removal of residue from grazing was assumed following harvest. EF_1, the emission factor for the emission of N_2O from N inputs, was taken to be 0.005 as per default value for a dry climate (IPCC, 2019). F_{PRP} is the kg N in urine and dung deposited by sheep and was calculated based to the typical mass of the sheep according to the farm manager and the IPCC default factor for sheep in Africa. EF_{PRP}, the emission factor for N_2O emissions from N deposited by grazing animals was taken to be 0.003 as per default value for sheep in Africa (IPCC, 2019).

2.6. Statistical analyses

Statistical analyses were undertaken in statistical software R version 4.2.1 (2022). Analyses of variance (ANOVAs) followed by Tukey's Honest Significant Difference post-hoc tests were performed to assess differences in rotation system for responses of soil bulk density, combined silt and clay contents, yield, protein content, and absolute and relative biomass. Assumptions for ANOVAs were tested with a Shapiro-Wilk normality test and a Bartlett test of homogeneity of variances. A Kruskal-Wallis rank sum test was used to analyse differences in clay content and wheat protein content across rotations, as data was not normally distributed. A Welch ANOVA was used for wheat yields when samples were heteroscedastic. Pearson's Chi-squared test was used to test associations between rotation system and coarse fraction classes (all expected cell frequencies were greater than 5). The 'emmeans' package 1.8.0 (Lenth et al., 2022) was used to model linear regressions for long-term soil organic C, C stocks, and protein content. For N_2O and CH_4 fluxes, 'nlme' (Pinheiro et al., 2024) and 'multcomp' (Hothorn et al., 2022) packages were used to define linear models for rotation and crop type. Multiple contrast tests were then conducted to compare the level of the influence factor. The coefficient of determination from the linear regression of in $\mu l\, l^{-1}\, h^{-1}$ N_2O time was used for quality control, zeroing fluxes where $R^2 < 0.6$. As CO_2 concentrations logically increased over time in the chamber, CO_2 fluxes were used as plausibility checks and to resort vials if a sorting error was assumed. Statistical significance of factors was declared at $p \leq 0.05$.

3. Results

3.1. Soil carbon sequestration rate

All systems showed increasing soil organic C with high variation across years and plots, but neither soil organic C nor the slope increase was different ($p > 0.05$) across rotations systems. Soil organic C values in 2000 vs. 2021 were WCWL: 1.04 % vs 1.43 %; WWWC: 0.94 % vs. 1.39 %; and WMCM: 0.92 % vs. 1.38 %.

Similarly, soil C stocks slopes were not different across systems (p > 0.05), thus no system differed in sequestration potential (Fig. 1). There was no difference (p > 0.05) in bulk density across rotations, but WMCM correlated ($\chi^2 = 56.25, p < 0.05$) to a smaller coarse fraction. Analyses of physical soil parameters: bulk density, coarse fraction, and soil texture are in Supplementary Table A.5 and Fig. A.2, and soil C stock variation in Supplementary Fig. A.7.

3.2. Seasonal nitrous oxide and methane fluxes

Nitrous oxide fluxes were low, variable and had no differences (p > 0.05) between rotation systems (Fig. 2A). Similar studies in Mediterranean croplands with low N fertiliser inputs also found N_2O emissions to be low (Cayuela et al., 2017; Manalil et al., 2014; Shumba et al., 2023; Tellez-Rio et al., 2015).

The only significant emission response observed was an N_2O emission spike in December in systems with legumes (WCWL, WMCM), highlighting rotation differences (Fig. 2B). The spike coincided with a sampling event that occurred the day following 11.6 mm of precipitation (See Supplementary Fig. A.1 for seasonal precipitation). Other precipitation events of 11.6 and 12.4 mm occurred prior to sampling in June and July, respectively, but did not increase emissions. See Supplementary Figs. A.3 and A.4 for detailed emission fluxes at a sub-rotation level.

The N_2O fluxes determined via in-field measurements differed significantly from results calculated via the IPCC Tier 1 methods, which resulted in 0.65, 0.64 and 0.53 kg N_2O-N ha^{-1} $year^{-1}$ for WWWC, WCWL, and WMCM, respectively.

Methane emissions overall were negligible, similar to literature (Barton et al., 2013), and did not differ (p > 0.05) (Fig. 2C). Slightly more emissions occurred over the spring period, where warmer temperatures and water availability are ideal for microbial activity, potentially depleting soil oxygen quicker than replacement by diffusion, creating temporary anaerobic conditions (Fig. 2D). The net positive cumulative emission of CH_4 found in the WWWC treatment arose from one plot, while the remainder were net negative.

Manure releases CH_4 and N_2O, but no increased fluxes were detected which corresponded to grazing events. Possibly stocking rate was too low to cause a response, or the soil collar discouraged excretion within the measurement area. However, the contents within the collar were grazed to the same level as the plot.

3.3. Production performance

Across 2021 (Fig. 3A, C, E), all crop rotations differed ($p < 0.05$) in relative biomass production: WMCM > WCWL > WWWC (Fig. 1A). In both 2021 and over the period 2000–2021, WMCM's wheat and canola phases performed better than WWWC, and wheat performed better in WCWL than WWWC (p < 0.05) (Table 1). Over the period 2000–2021, WMCM consistently produced higher yields, followed by WCWL, except for dry years, where all systems fared poorly (Fig. 1B, D, F). When normalising to a relative yield, based on the maximum yield achieved that year for each crop type, WWWC, WCWL, and WMCM, achieved a relative yield of 0.72 (SE = 0.012, $n = 167$), 0.81 (SE = 0.012, $n = 125$), and 0.87 (SE = 0.017, $n = 84$), respectively.

The protein content of wheat grain followed the pattern WWWC < WCWL < WMCM and was different (p < 0.05) for all rotations across 2000–2021 and 2016–2021, but not in 2021, where systems had

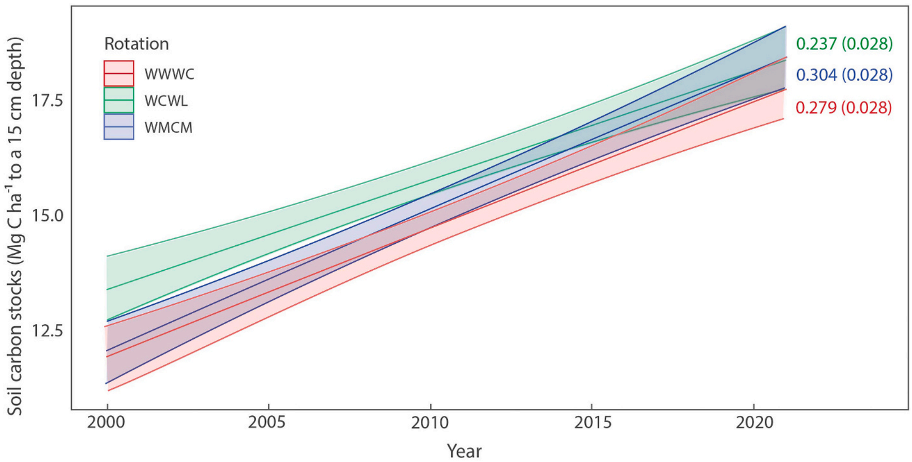

Fig. 1. Changes in soil C stocks over the experimental period. Graphical representation of soil C stocks accumulation over 21 years for different crop rotation systems, indicated by colour with a 95 % confidence interval band. An upward trend is visible in all rotation systems. Carbon stocks were calculated using soil organic C values taken annually, coarse fraction volume, and bulk density, representing the accumulation in the top 15 cm of soil depth. Linear regressions were modelled using plot as a blocking factor. Slopes displayed with standard error were not different ($p > 0.05$). Treatment abbreviations for rotation systems: wheat-wheat-wheat-canola (WWWC), wheat-canola-wheat-cover crop/lupin (WCWL), wheat-medic pasture-canola-medic pasture (WMCM).

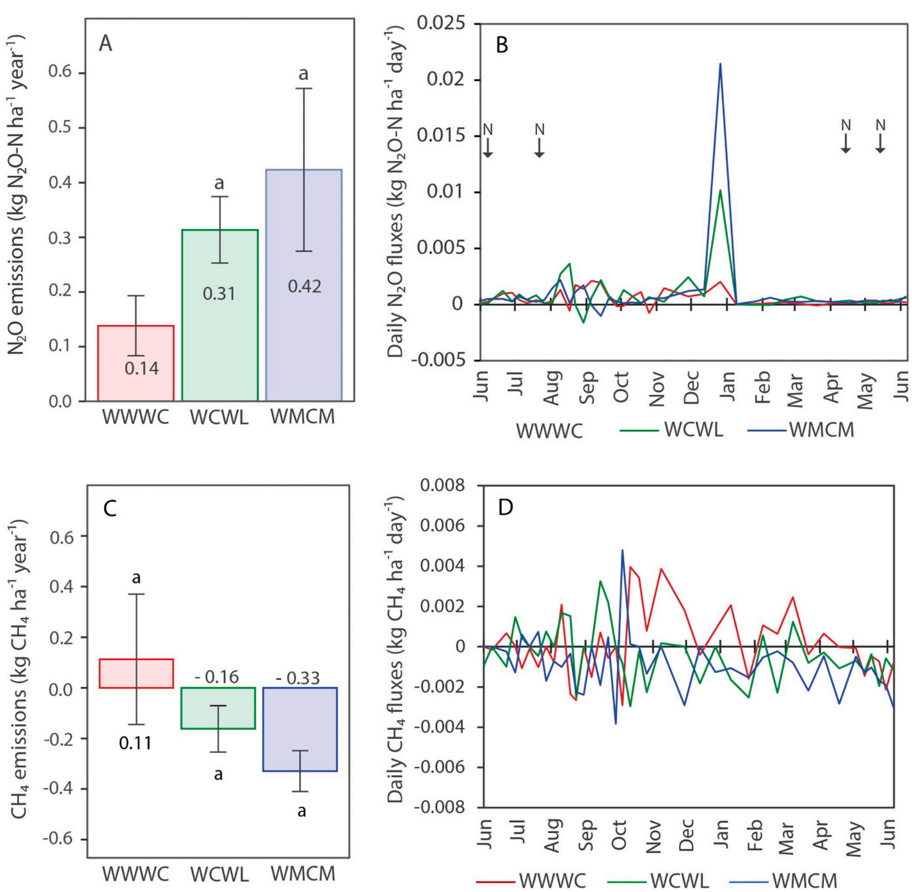

Fig. 2. **(A)** Total annual N_2O emissions for crop rotation systems. Error bars depict standard error; different letters depict differences ($p < 0.05$). **(B)** Daily N_2O emissions for crop rotations measured via the static closed chamber method from 3 June 2021–8 June, 2022. Nitrous oxide emissions remain at low background levels over the winter growing season (May – November). Still, a large flux contributing majority of the total emissions occurred in the summer fallow in response to out of season rainfall. For the single flux, standard errors are depicted by error bars. Applications of N fertiliser throughout the growing season is indicated with grey arrows. **(C)** Total CH_4 emissions across rotation systems. Error bars depict standard error; different letters depict differences ($p < 0.05$). **(D)** Daily CH_4 emission fluxes for crop rotations measured via the closed chamber method from 3 June 2021–8 June 2022. The quantity of emissions was not different across rotations ($p > 0.05$) and tended to be negative, which is typical of semi-arid dryland systems. **(A-D):** Treatment abbreviations for rotation systems: wheat-wheat-wheat-canola (WWWC), wheat-canola-wheat-cover crop (WCWL), wheat-medic pasture-canola-medic pasture (WMCM).

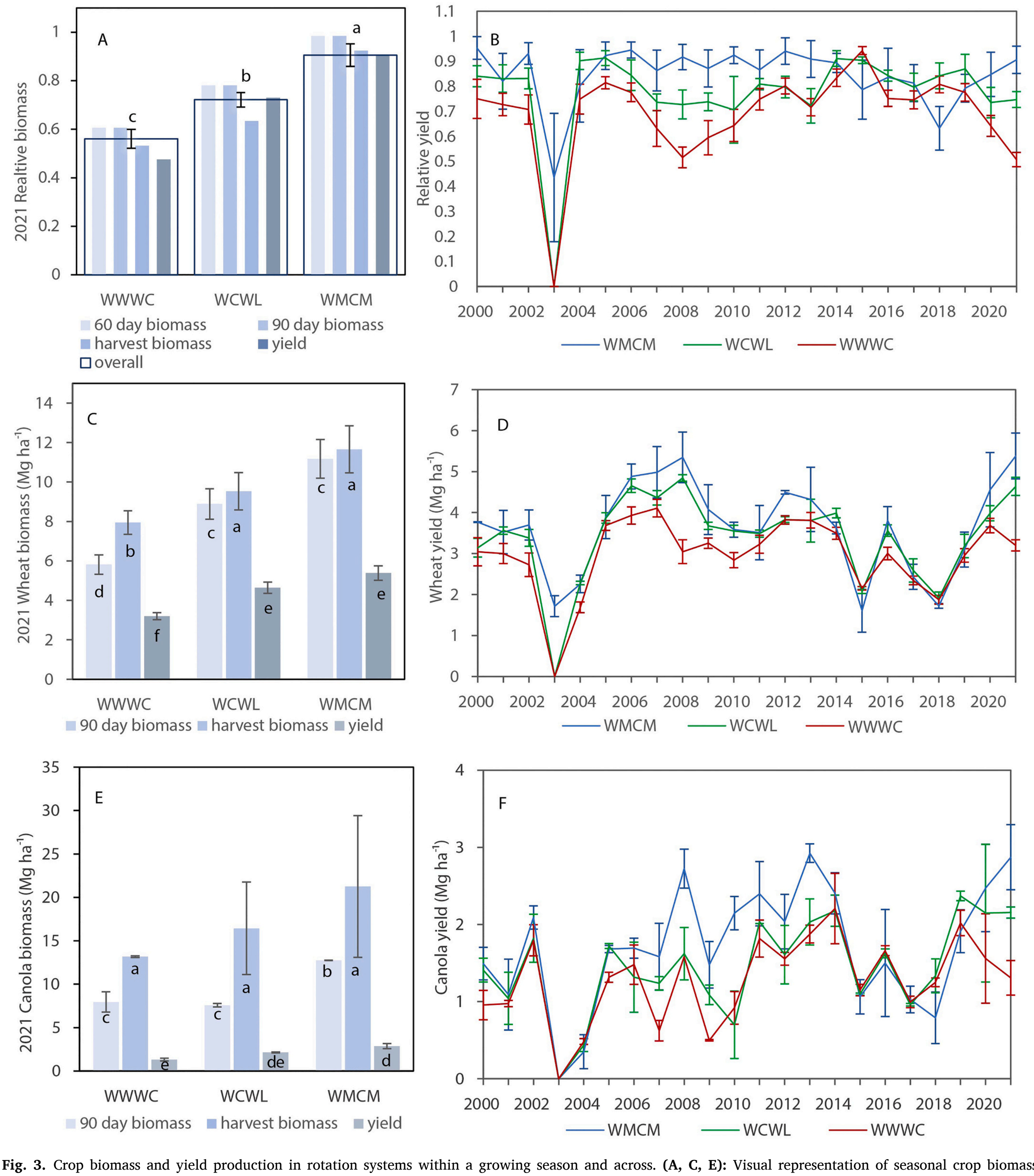

Fig. 3. Crop biomass and yield production in rotation systems within a growing season and across. **(A, C, E):** Visual representation of seasonal crop biomass production, yield with sampling occurring throughout the 2021 growing season for relative biomass production of both wheat and canola **(A)**, wheat **(C)**, and canola **(E)**. For **(C)** and **(E)** error bars and letters indicate differences within each measurement type. **(B, D, F):** Average cash crop yields between 2000 and 2021. Years 2003–2004 were very dry years and a drought occurred over 2015–2017. In 2003, all treatments failed to yield any outputs except for WMCM wheat plots. The average relative yield per hectare for all cash crops **(B)**, the absolute average yield per hectare for wheat **(D)** and **(F)** canola. Different superscript letters denote differences ($p < 0.05$). Error bars represent standard error. **(A – F):** Treatment abbreviations for rotation systems: wheat-wheat-wheat-canola (WWWC), wheat-canola-wheat-cover crop/lupin (WCWL), wheat-medic pasture-canola-medic pasture (WMCM).

Table 1
Canola yield, wheat yield, and wheat protein content data summary for 2001–2021 (the entire experimental period), 2016–2021 (the period in which the L phase in WCWL represents a cover crop mixture) and 2021. Separate statistical analyses were conducted for each period and response; differences ($p > 0.05$) are denoted by different superscript letters. Treatment abbreviations for rotation systems: wheat-wheat-wheat-canola (WWWC), wheat-canola-wheat-cover crop/lupin (WCWL), wheat-medic pasture-canola-medic pasture (WMCM).

Period	2000–2021		2016–2021		2021 only	
	Canola yield (Mg ha $^{-1}$)	Sample size	Canola yield (Mg ha $^{-1}$)	Sample size	Canola yield (Mg ha $^{-1}$)	Sample size
WWWC	1.33 (0.10) ^{b}	41	1.47 (0.18) ^{a}	12	1.31 (0.20) ^{b}	2
WCWL	1.52 (0.13) ^{ab}	42	1.77 (0.26) ^{a}	12	2.15 (0.28) ^{ab}	2
WMCM	1.80 (0.13) ^{a}	42	1.76 (0.26) ^{a}	12	2.85 (0.28) ^{a}	2
	Wheat yield (Mg ha $^{-1}$)	Sample size	Wheat yield (Mg ha $^{-1}$)	Sample size	Wheat yield (Mg ha $^{-1}$)	Sample size
WWWC	3.09 (0.08) ^{b}	126	2.84 (0.16) ^{a}	36	3.20 (0.18) ^{b}	8
WCWL	3.54 (0.13) ^{a}	83	3.30 (0.25) ^{a}	23	4.64 (0.29) ^{a}	6
WMCM	3.64 (0.16) ^{a}	44	3.50 (0.31) ^{a}	12	5.38 (0.36) ^{a}	4
	Wheat protein content %	Sample size	Wheat protein content %	Sample size	Wheat protein content %	Sample size
WWWC	11.4 (0.13) ^{c}	126	10.6 (0.27) ^{c}	36	8.8 (0.36) ^{a}	6
WCWL	11.5 (0.21) ^{b}	83	11.5 (0.43) ^{b}	23	10.0 (0.57) ^{a}	4
WMCM	12.6 (0.26) ^{a}	44	12.6 (0.54) ^{a}	12	10.7 (0.72) ^{a}	2

unusually lower protein contents (Table 1). Over 2000–2021, protein content has dropped in WWWC, to a lesser extent in WCWL, but not in WMCM (Fig. 4A). Slopes were not different ($p > 0.05$) between rotations, but rotation system, plot, and year affected ($p < 0.05$) the linear model. From 2016 to 2021, 71 % of wheat from WWWC was bread grade, while this proportion was 90 % for WLWC and 89 % for WMCM Republic of South Africa. Department of Agriculture Forestry and Fisheries (2019). In 2021, the protein content of WWWC was very low at 8.8 %; one out of six wheat plots made the bread grade. Incorporating legumes proved an essential supply of N: WMCM exported 50 % more N in grain than WWWC while receiving 50 % less fertiliser over 2016–2021 (Fig. 4B). Protein content and N exported are indicators of N availability, a needed reactant for N_2O formation in the soil.

4. Discussion

4.1. Soil organic carbon and historical carbon debt

Different responses of rotations systems can be explained by the long-term deposition of crop residues following different cropping phases. Sun et al. (2020) found no-till to enhance sequestration compared to using tillage in warm, arid climates, and sequestration was influenced more by C and N inputs than rotation diversity or legume incorporation. Canola and wheat produce biomass with high C:N and lignin:N ratios making these tissue inputs slower to decompose (Lemaire et al., 2014; Reinsch et al., 2021). Alternatively, annual medics decompose faster (Cooper et al., 2017) but supply N, which is important for the stabilisation of soil organic C (Angeletti et al., 2021). Jian et al. (2020) found biomass production was positively correlated while C:N ratio negatively correlated with soil C increases, so while C inputs are necessarily related to increases in soil C, lower C inputs but with a smaller C:N ratio may be just as effective for sequestering carbon as more C inputs with a higher C:N ratio. This indicates how the lower biomass-producing medic phase resulted in WMCM accumulating C at a similar or even higher rate than other rotations despite residue removal through grazing. Carbon stock accumulation is difficult to detect statistical significance due to high natural variation, so the non-significant differences remain valuable in understanding management effects (Kravchenko and Robertson, 2011). The increase in soil C stocks in the upper soil layer across all rotations possibly resulted from the switch from intensive tillage to conservation agriculture in 1997, although there is no control to confirm this.

A limitation was possible underestimation of C stocks due to sampling to 15 cm depths, while sequestration occurs deeper at this site (Cooper et al., 2017). Estimating sequestration potential assumes a steady trajectory, but with C stocks having limits, this rate will decline. Continued investigation is necessary to determine any system differences at C steady-state. Carbon sequestration is not a true emission offset due to its finite ability to store C, reversibility back to CO_2, and the recognition of the soil C stocks of its pre-cultivated natural state. Arguably, C is sequestered relative to a prior degraded state, that repays a historic C debt acquired in the initial conversion to cropland (Fig. 5). The initial move towards no-till in the 1980s in the Western Cape was a response to severe soil erosion, as intensive cultivation degraded the soil (Strauss et al., 2021). Present-day estimates of soil organic C content for the undisturbed Swartland Renosterveld areas are 1.7–1.8 % (Mills et al., 2013; Swanepoel and Tshuma, 2017). Carbon accumulation occurs but remains lower than its potential state (Fig. 5). The current sequestration rate must remain constant for another 14–20 years to increase current C stocks by 3 to 6 Mg C ha-1 to reach the pre-cultivated regional estimate. However, this will not occur if the C steady-state is lower for croplands than for undisturbed lands.

4.2. Yield and protein content

There were production differences for grain yield and quality, where WWWC had the lowest yields and quality and WMCM the highest. Yields per ha indicate production capacity, and although yields per ha were lowest in WWWC, it had the highest component of wheat within its rotation (75 %), with WCWL and WMCM containing 50 % and 25 % wheat, respectively (Supplementary Table A.6 summarises total tonnes of system outputs). In 2021, the WWWC system produced the most wheat compared to the other rotation systems, but the least bread-grade wheat, due to poor protein content, downgrading the majority to animal feed, showing that even though wheat formed the biggest component of the rotation, the system was still not optimal for wheat production. Systems WCWL and WMCM benefited from additional N input via biological fixation and animal excreta, while WWWC was wholly reliant on fertiliser application which may be insufficient, throttling the plants' ability to produce protein. Over time, lower residues in WWWC and lower protein levels result in less organically bound N than WCWL and WMCM.

Points in the season where moisture availability coincides with heat accelerate plant growth and microbial activity. This rapid growth requires nutritional support, and if not supplied by the increased metabolism of organic matter, microbes outcompete plants (Chen et al., 2021). In soils with high C:N organic matter, characteristic of cereal-

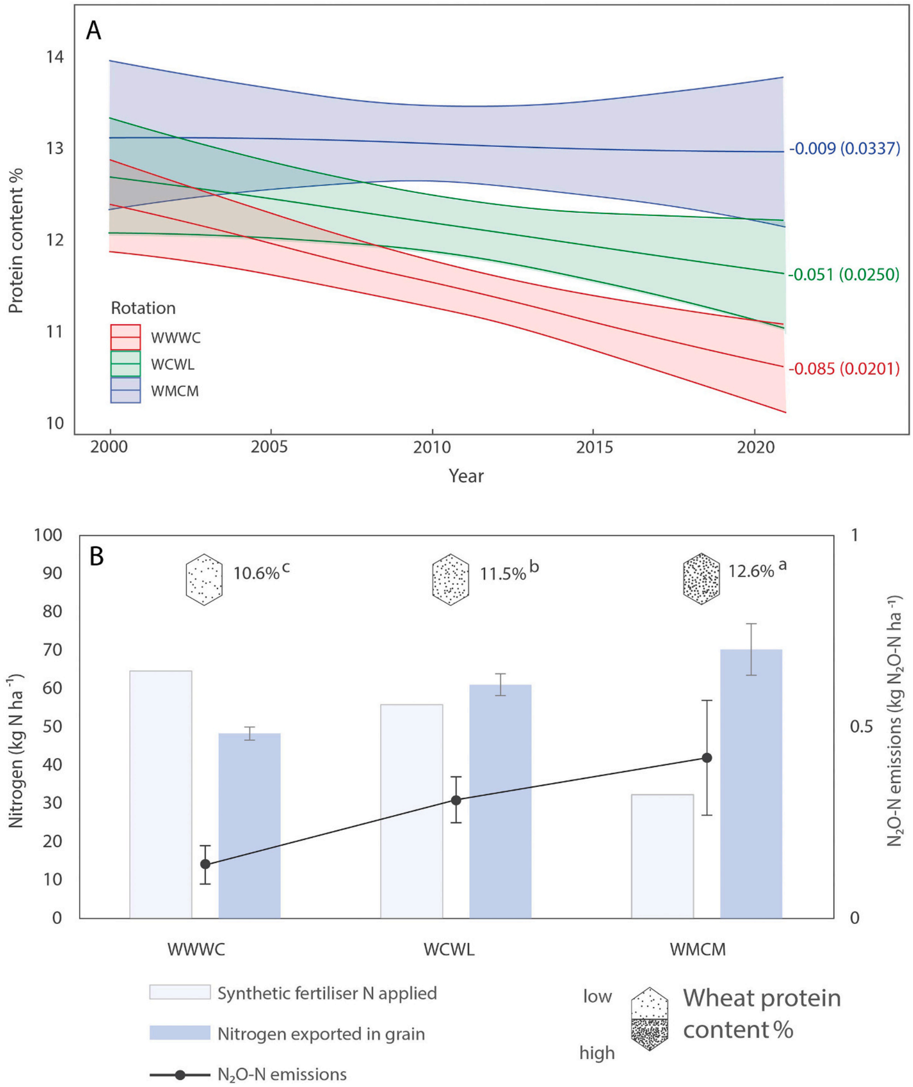

Fig. 4. Wheat protein content and N balance in various crop rotation systems.
(A): Estimated linear change in wheat protein content, depicted with slope and standard error brackets. Protein content is an important parameter in the bread wheat grading system, where protein contents of below 9.5 % result in downgrading to class other. **(B):** Visual summary of the annual nitrogen balance and the role of N fixation in different crop rotation systems over the period 2016–2021. Nitrogen exported was calculated by multiplying the yield by protein content by the amount of N in crude protein. Rotation WMCM had higher yields, protein contents, the lowest fertiliser applications. Total N_2O emissions were not significantly different. The difference between N export and fertiliser N applied in WCWL and particularly in WMCM represents the effect of N fixation during the legume phases. Error bars represent standard error. **(A-B):** Treatment abbreviations for rotation systems: wheat-wheat-wheat-canola (WWWC), wheat-canola-wheat-cover crop/lupin (WCWL), wheat-medic pasture-canola-medic pasture (WMCM).

dominated systems, N becomes limiting and immobilised during organic matter breakdown (Korsaeth et al., 2002; Ashiq et al., 2021). Through long-term legume incorporation and manure inputs, C:N is lower and releases N into the system when metabolised. This improved the partitioning of N to the crop over the season, especially in the critical grain filling stage (Maali and Agenbag, 2003). As soil organic material increases and soil disturbance is minimised, soil biota is better supported (Morugán-Coronado et al., 2022), emphasising rotation differences in N availability at critical points.

4.3. Soil nitrous oxide emissions

Conditions of soil moisture, soil temperatures >25 °C, and surplus N also promote N_2O production (Barton et al., 2008). Off-season rainfall, in the absence of plant growth, led to N_2O formation which was correlated to N availability in the soil from the incorporation of legume phases. This spike uniquely occurred in the warmer fallow season, after a pronounced wet-dry cycle, with no possible plant N uptake. Similarly, Barton et al. (2008), found the majority of N_2O emissions occurred following summer rainfall over the fallow period. Higher N_2O spikes occurred after rewetting dry soil (Ruser et al., 2006), with legume plots having a more intense response than wheat plots (Manalil et al., 2014). The high flux emission spike in response to rewetting would have continued for 24 h so the impact may be overestimated due to the sampling interval (Barton et al., 2008; Manalil et al., 2014). Alternatively, three significant fallow-rainfall events (precipitation >10 mm) were potentially missed as they occurred multiple days prior to sampling. Chen et al. (2021) found that crop residue inputs increased N_2O emissions, but decreased with an increasing C:N ratio. It was also observed in canola phases in WMCM, as the canopy opened during pod-filling, medics emerged, lowering the C:N ratio or crop residues left behind even in non-legume years. This trial was fertilised conservatively and included the use nitrification inhibitors with some application, which reduced the potential of N_2O emissions throughout the growing

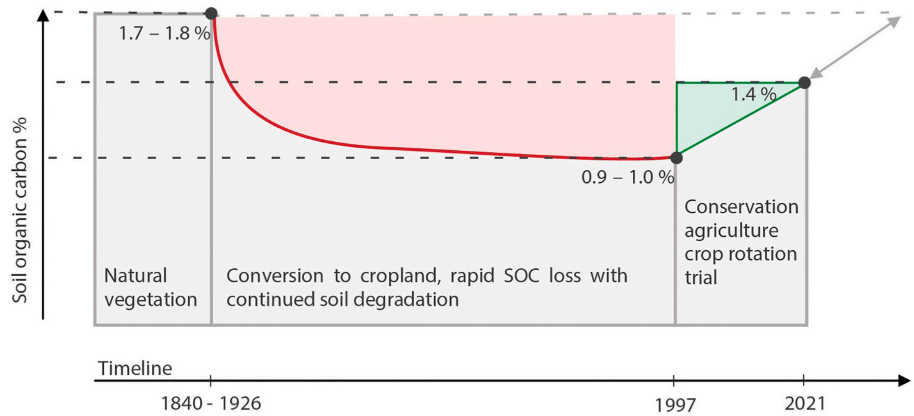

Fig. 5. Changes in soil C stocks contextualised over a longer timeline and land use change. Visual illustration of historical soil organic C changes at the trial site. The legacy of C loss from land use change remains even after 25 years of C sequestration into soils. Soil organic C content in the pre-cultivated natural site is estimated to be 1.7–1.8 % (Mills et al., 2013; Swanepoel and Tshuma, 2017). Transformation into cropland sometime between 1840 and 1926 likely resulted in a significant loss of C through ecosystem disturbance, followed by a slower continual loss due to wheat monoculture and conventional tillage; this is stylised by the red curved line for illustrative purposes and is not based on empirical data. In 1997, in response to the need to investigate conservation agriculture practices, the conservation agriculture trial commenced, and annual soil organic C sampling took place on this site. Although organic C content has increased by ±45 %, a historical C debt remains. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

season (Shcherbak et al., 2014) but had no impact of the fallow-season emission hotspot. Climate change is predicted to increase summer rainfall in the Western Cape which would increase N_2O emissions. This is further complicated as climate-smart agriculture aims to build soils to increase the mineralisation fraction of organic matter to provide nutrients, retain more water and improve yields. A limitation of these results is high variability that was increased by the soil's lateral drainage, the nature of soil N_2O formation and emission as short-lived bursts under specific conducive conditions and only one significant N_2O emission flux event. Negative N_2O fluxes occurred haphazardly. Although reduction of N_2O to N_2 is possible (Chapuis-lardy et al., 2007; Tellez-Rio et al., 2015), this is neither significant nor manageable (Robertson, 2004) and this activity is likely experimental noise and an artefact of near zero-fluxes rather than a sink.

The IPCC Tier 1 calculation method makes use of global default emission factors with large uncertainty ranges (Fig. 6A), while using more accurate Tier 2 and 3 methods requires rigorously documented country-specific emission factors. The lack of many regional datasets on N_2O emissions adds difficulty around reducing cropland N_2O reduction, as emission fluxes are responsive to site-specific conditions. In this case, disaggregating emission factors by only wet vs. dry climates would miss a flux resulting from a wet-dry cycle occurring over the fallow period, which may be an important detail for N_2O reduction in croplands under a Mediterranean climate regime.

4.4. Soil greenhouse gas balance

Many crop rotation outcomes are interlinked (Fig. 6B). Dropping protein contents poses significant problems for the profitability and sustainability of the cropping system and the crop-legumes-livestock rotations resulted in improved wheat protein by providing additional N sources and possibly influencing the provisions of N to crops from the soil organic fraction. Legume and livestock incorporation improved yield performance, reduced fertiliser reliance, and increased protein content, which contextualised the increased N_2O emissions. Fertiliser N reliance is also an important aspect of system sustainability due to the carbon-intensive Haber-Bosch process of producing N and it's transport, although these emissions do not occur on-farm, management decisions can result in significant additional emissions elsewhere (Osorio-Tejada et al., 2022). Soil GHG balance is only one component of global warming potential as livestock produce $CH_{4,}$ and machine passes release fossil GHG emissions, and commodities go into different value chains.

When compared via the GWP100-AR6 metric, annual C soil accumulation sequestered more CO_2-eq than the soil emissions of N_2O and CH_4 (Fig. 6A). Based on the relative size of the CO_2-_eq removal it provided, the return of C to soils over the experimental period resulted in a negative soil GHG balance for all systems. Carbon sequestration into soils is not a true emission offset due to impermanence and a finite storage capacity, secondly emitted N_2O has no significant sink and cannot be abated once emitted. Although N_2O and CH_4 emissions occurred at similar orders of magnitude, the difference in CO_2 equivalence between these GHGs meant that even trace amounts of N_2O were important while there was neither a significant sink nor source of CH_4. Net soil GHG emissions were not different as neither N_2O, $CH_{4,}$ nor C sequestration differed.

5. Conclusion

Crop rotation is a relatively adjustable component in an agricultural system. Its design has significant implications for management practices and possibly the soil processes regulating GHG emissions and sequestration. Annual C sequestration rates and soil fluxes of CH_4 and N_2O were not different between rotations. However, rotations responded differently to a summer rainfall event in the fallow season. A spike in N_2O emissions occurred at an intensity proportional to the number of legume/livestock phases in a rotation. Systems that integrated livestock and/or legumes consistently outperformed the cash crop-only rotation system in yield, biomass production, and protein content of wheat while being far less reliant on fertiliser. The legumes and livestock in the rotation brought significant improvements to production. Although the cash crop-only rotation had the lowest N_2O emissions, the dropping protein content indicates that this rotation with its current N fertilisation is unsustainable for the long-term production of bread wheat, due to the poorer food output at an increased usage of carbon-intensive fertiliser N inputs. The authors assume the same mechanism underlies production performance and N_2O emissions: microbial activity released N via mineralisation systems with a legacy of low C:N leguminous residues and manure but immobilised N in the cereal-dominated system with a legacy of high C:N residue inputs. The hotspots for N_2O emissions lie in the off-season, making reduction difficult as minimal management activities occur over this time compared to in-field measurements. There is a significant challenge in designing N_2O mitigation strategies due to the

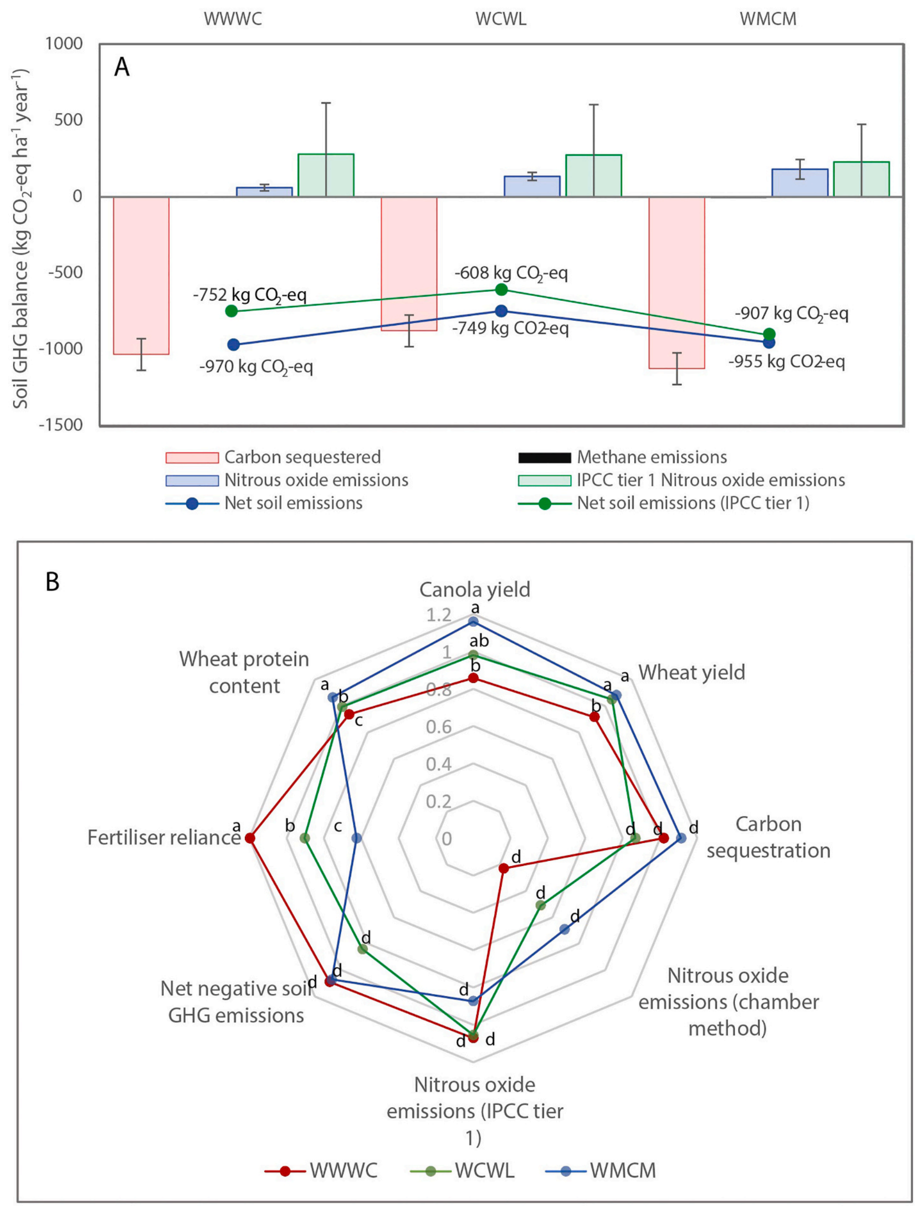

Fig. 6. **(A)** Annual soil GHG emission balance, a relative comparison of emission and sequestration of GHGs, including a comparison between methods of N_2O determination. Conclusions on systems should be interpreted with caution as neither C sequestration, N_2O emissions nor CH_4 emissions were different ($p > 0.05$). In-field N2O fluxes, measurements determined via the closed static chamber method are indicated in blue. The IPCC's Tier 1 method of N_2O determination from managed croplands is shown in green, with the error bars representing the uncertainty range. Net soil emissions are the net CO_{2-}eq of the C sequestered into soils and the CH_4 and N_2O emitted over the experimental year, distinguishing between when the closed static chamber method, compared to IPCC Tier 1 values, was used. The difference corresponds to a difference of 218, 142 and 50 kg CO_2-eq per ha for WWWC, WCWL, and WMCM, respectively. **(B)** Radar graph comparing important trade-offs across crop rotation systems: yield, wheat protein content, fertiliser reliance (kg N applied per kg N exported), soil C sequestration, N_2O emissions as determined through direct chamber measurements vs. IPCC tier 1 values, and net negative GHG emissions over the experimental period (direct CH_4 and N_2O emissions, minus C sequestered). Variables were based on the experimental period of 2000–2021, except N_2O emissions and net negative GHG emissions, which relied on data from 2021 only. Different letters depict differences ($p < 0.05$) for each response. All variables were normalised based on the average response, except for N_2O (chamber), which was normalised by the same factor as N_2O (IPCC) to ensure comparability. **(A-B):** Treatment abbreviations for rotation systems: wheat-wheat-wheat-canola (WWWC), wheat-canola-wheat-cover crop/lupin (WCWL), wheat-medic pasture-canola-medic pasture (WMCM). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

lack of existing N_2O flux datasets, which are needed to develop specific regional emission factors for higher tier estimation methods. More diverse rotations, particularly when legumes formed part of the rotation sequence performed better in terms of yield and quality over 20 years. All systems increased soil carbon stocks over the experimental period, regardless of crop rotation.

CRediT authorship contribution statement

Lisa Matthews: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Johann A. Strauss:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Data curation. **Thorsten Reinsch:** Writing – review & editing, Validation, Resources, Investigation. **Hendrik P.J. Smit:** Writing – review &

editing, Validation, Resources, Formal analysis. **Friedhelm Taube:** Writing – review & editing, Supervision, Resources. **Christof Kluss:** Writing – review & editing, Validation, Formal analysis, Data curation. **Pieter A. Swanepoel:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.agsy.2024.104218.

Data availability

Data will be made available on request.

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