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Faba bean introduction makes protein production less dependent on nitrogen fertilization in Mediterranean no-till systems

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ABSTRACT

Context: Under Mediterranean rainfed areas, no-till cereal-based systems have been adopted to cope with water availability and increasing input costs. However, the increased risk of biotic stresses, high N-fertilizer dependence, and current EU policies warrant cropping systems re-design.
Objective: Evaluate diversification and N fertilization as strategies to improve N use efficiency at the cropping system level and quantify its productivity.
Methods: Four crop sequences combined with four levels of N fertilization were assessed in a three-year field experiment in semiarid rainfed north-eastern Spain. Crop sequences were continuous winter wheat (WCS) and three-year diversified rotations with pea (PCS), faba bean (FCS), or a multi-service cover crop (MSCS) and two years of cereals. Crop, pre-crop and cropping system levels were considered. Agronomic evaluation included crops above-ground biological N fixation (Ndfa), net N balance (Ndfa minus N removed by grain), soil N mineralisation productivity, energy to N tradeoff (ENT), and N use efficiency of protein (NUEp) production.
Results: Pea yields ranged from 0 to 766 kg ha^{-1} and Ndfa from 24% to 54%. Faba bean yield ranged from 1378 to 4251 kg ha^{-1} and Ndfa from 32% to 72%. Net N balance was close to neutral for pea while in faba bean it ranged from 41 to −21 kg N ha^{-1}. Alternative pre-crops led to greater soil N mineralisation (51 kg N ha^{-1}, on average) and higher wheat yield (564 kg ha^{-1}, on average) compared to wheat as the pre-crop. N fertilization increased protein yields, with FCS presenting the highest yields at all N fertilizer rates. This effect led to a stable NUEp (1.69 kg protein kg N supply^{-1}), as the protein yield increased proportionally to N supply.
Conclusions: Diversification improved the succeeding wheat performance and grain legumes N fixation exceeded grain N removal. Introducing legumes into cropping systems led to a decrease in energy productivity compared to the cereal-based system. However, protein production in the FCS was higher than in any other cropping system regardless of the N fertilizer rate.
Implications or significance: Crop diversification adds challenges and risks in dry Mediterranean areas. However, the study shows that crop diversification with faba bean can decrease cropping system's N-fertilizer dependence and increase protein productivity, contributing to cropping systems' sustainability.

1. Introduction

Rainfed cropping systems dominate crop production worldwide, representing about 80% of global agricultural land use (Hatfield et al., 2001). Under Mediterranean conditions, water availability is the main limiting factor for crop productivity, mainly due to terminal drought and high temperatures that compromise the grain filling period (Loomis and Connor, 1992). Therefore, strategies aimed at increasing soil water storage and improving water use efficiency are a path towards more sustainable and productive cropping systems (Passioura and Angus, 2010). In some Mediterranean areas, such as the Ebro valley in Spain, continuous no-till systems have been adopted as an adaptation strategy to dry conditions with successful outcomes such as increased soil water storage (Lampurlanés et al., 2016), higher cereal yields (Angás et al., 2006) and reduced costs associated with fuel, labour and machinery (Bashour et al., 2016). Rainfed no-till systems are largely based on winter cereals, given their adaptability and profitability to such conditions (Kirkegaard et al., 2014). However, continuous no-till cereal

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production can increase the risk of some biotic stresses, such as severe infestations of grass weeds (e.g. *Bromus diandrus* Roth (García et al., 2014)), fungi diseases (e.g. Helminthosporium leaf blights) and insects such as *Zabrus tenebrioides* (see Plaza-Bonilla et al., 2017a). The management of biotic stresses is increasingly complicated, especially under no-till systems, where dependence on a few active ingredients and limited management alternatives are available (Kirkegaard et al., 2014). Crop diversification can be a lever to alleviate some of these problems and might contribute to the sustainability of such systems in the long run (Smith et al., 2023). The actual agronomic and environmental problems in farming, and policy rules in the case of Europe (European Commission, 2021), require farmers to redesign their cropping systems. Introducing legume crops into cropping systems is a step towards diversification and can bring different agronomic benefits such as (i) the supply of N through biological fixation (Peoples et al., 2009), (ii) the potential for N fertilisation rate reduction in the following crop (Plaza-Bonilla et al., 2017c), and (iii) a yield increase in the following crop (Angus et al., 2015; Preissel et al., 2015). The positive effects of legumes to the succeeding crops are known as the pre-crop effect, which can be divided into the N credit effect and the break crop effect (Chalk, 1998; Notz and Reckling, 2022). The former one relates to the possible N availability increase after growing a legume crop, provided that N biologically fixed is greater than N exported in grain (also known as the net N balance). The latter refers to the yield increases non related to nutrient supply such as pest, weed and disease control, soil quality, etc. (Bennett et al., 2012). Despite the benefits listed above, crop rotations with legume crops also lead to challenges and are often associated with unstable yields compared to winter-sown cereals (Fletcher, 2019; Reckling et al., 2018).

Another option to cope with the limitations of cereal-based cropping systems is the use of fallow. Under semiarid Mediterranean conditions, long fallows (>12 months) have been extensively used to increase water storage and nutrient availability, as well as to reduce inputs and machinery costs (Cann et al., 2020; O'Connell et al., 2002), to increase the following cereal yields. Traditionally, fallow land has been kept bare by tillage or chemical weed control increasing the risk of soil erosion (Whish et al., 2009) and reducing the C input to the soil (Tiefenbacher et al., 2021). A possible alternative is a multi-service cover crop (MScc) based on spontaneous vegetation and managed by cutting and/or chopping before the weed seed set, which would naturally provide ecosystem services (biodiversity, soil cover, etc.) entailing low costs (Zinngrebe et al., 2017). While these non-productive areas can bring environmental benefits (Zinngrebe et al., 2017) and are encouraged by policy (European Court of Auditors, 2017), they come at the expense of lower land productivity, which directly affects the farmer's profit (Chen et al., 2023).

Changes in input costs (namely, synthetic N fertiliser), and fewer available and effective pesticides are other drivers to foster crop diversification. For instance, urea prices increased from 169 € t^{-1} in 2000–489 € t^{-1} in 2021 in Spain (values refer to yearly average expressed as current prices) (MAPA, 2022). The steady increase in production costs affects the profitability of such cropping systems, especially if these trends should continue in the future (Baffes and Koh, 2022). Furthermore, the use, and sometimes overuse, of N fertilisers in agriculture leads to potential environmental problems such as higher N_2O emissions, groundwater N pollution, etc. (Plaza-Bonilla et al., 2014). In addition, they have an additional environmental cost associated with the synthetic N fixation through the Haber-Bosch process (Lal, 2004). Therefore, the present work focuses on the reduction of synthetic N fertilizer use with the introduction of legume crops or a MScc. Nonetheless, there are other alternatives that are already introduced such as rapeseed (*Brassica napus* L.) or being studied such as camelina (*Camelina sativa* L.).

Legume pre-crops increased the N use efficiency (NUE) of the following wheat crop in Mediterranean areas (López-Bellido and López-Bellido, 2001; Souissi et al., 2020). However, legume introduction effects extend further than the pre-crop effect, thus warranting a cropping systems assessment (Reckling et al., 2016). In that sense, legume crops under Mediterranean rainfed conditions have lower yields than cereals, making them unattractive for farmers (Fletcher, 2019; Robertson et al., 2010; Seymour et al., 2012). Nonetheless, they act as a sustainable N supply to the cropping system (Peoples et al., 2009). This N supply and the often lower yields of legume crops can impact the NUE at the cropping system level. While most studies studied diversification at the pre-crop level (e.g. Espinoza et al., 2015; Souissi et al., 2020), there is a lack of studies evaluating NUE at a multi-year level including all crop sequence phases. Therefore, it is necessary to study the NUE response to the interaction of crop diversification and N fertilization at the cropping system level. To achieve a fair comparison among cropping systems with different crop species, different indicators that account for the system's productivity are required (Costa et al., 2021). Given the energetic and protein nature of the crops grown in these systems, energy and protein yields can be used as productivity indicators at the cropping system level (Simon-Miquel et al., 2023).

Our objectives were to evaluate (i) the productivity of pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.) and their contribution to the soil-crop N balance through biological N fixation, (ii) the effect of pea, faba bean and a multi-service cover crop (hereafter referred as MScc) on the succeeding wheat combined with increasing N fertilizer rates and (iii) diversification and N fertilization as sustainable strategies to improve NUE at the cropping system level and quantify its main levers (productivity and N sources) under Mediterranean long-term no-till rainfed conditions. Our hyphothess were that (i) pea would outperform faba bean in terms of productivity but not in the amount of biologically fixed N, (ii) the introduction of an alternative crop would lead to smaller increases in the succeeding crop as the N fertilizer rate increases, and (iii) alternative cropping systems with grain legumes or MScc would lead to a reduction in energy productivity but an increase in protein yields.

2. Materials and methods

2.1. Experimental site and design

A field experiment was implemented in Selvanera (Spain, 41°49'52''N, 1°17'40''E, 465 masl) from 2019 to 2022. The area has a semiarid Mediterranean climate with an annual mean air temperature of 13.3 ºC and a total precipitation of 433 mm, distributed mainly in autumn and spring months (Fig. 1). Potential evapotranspiration is 1000 mm annually. Climate data was retrieved from a weather station located 3 km SE from the experiment owned by the Meteorological Service of Catalonia. The soil was characterized (0–30 cm) at the beginning of the experiment in October 2019 and was classified as Fluventic Haploxerept (Soil Survey Staff, 2014). Soil particle size distribution was 22.7% clay, 49.0% silt and 28.3% sand. Organic C and N (Kjeldahl) contents were 16.2 and 1.89 g kg^{-1}, respectively. Available P (Olsen) was 20 mg kg^{-1} and K (ammonium acetate) was 412 mg kg^{-1}. Electric conductivity (1:5) was 0.186 dS m^{-1}, pH was 8.2, carbonate calcium content was 300 g kg^{-1}, and bulk density was 1.5 g cm^{-3}. Water contents at the permanent wilting point and field capacity were 70 and 200 g kg^{-1}, respectively.

The field experiment consisted of a factorial combination of four crop sequences and four levelsof top-dressing N fertilization. The experimental design was arranged in a split-plot design with three replications. Crop sequence factor, placed in the main plots, consisted of continuous wheat (WCS), as a reference control for the area, and three alternatives consisting of a three-year rotation with pea (PCS), faba bean (FCS) and MScc (MSCS) (Fig. 2). In these crop sequences, three crops were involved, grain legume or cover crop, wheat as a following crop to the alternatives and barley (*Hordeum vulgare* L.) as the preceding crop to the alternatives. The inclusion of barley was a result of the cropping systems nature of this field experiment. The MScc was based on spontaneous

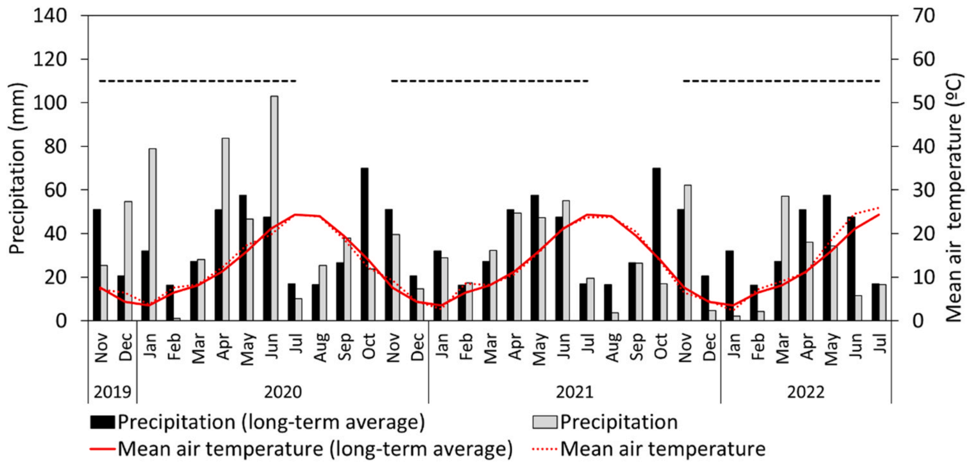

Fig. 1. Bagnouls and Gaussen diagram of the experimental site for the long-term average and the experimental period. Dashed lines at the top indicate the three experimental seasons.

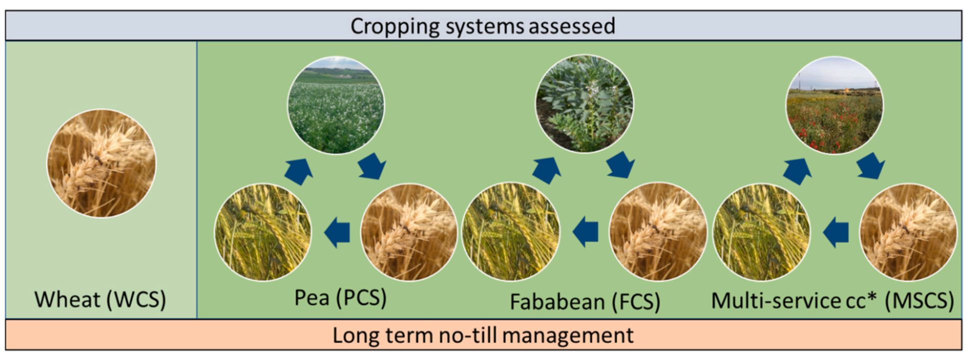

Fig. 2. Conceptual diagram of the four cropping systems assessed (reference system, in light green, continuous wheat (WCS); studied alternatives, in dark green, pea (PCS), Faba bean (FCS) and multi service cover crop (MSCS) cropping systems) combined with four levels of top-dressing N fertilization (0, 40, 80 and 120 kg N ha^{-1}, respectively) in the cereal crops. The alternative systems are based on a three-year rotation with the alternative crop (circles at the top within the darker green area) preceded by barley and followed by wheat (circles at the bottom). The experiment was placed in a long-term no-till field.

vegetation left to grow until the weeds' seed set began and then was controlled by chopping. Predominant species were *Papaver rhoeas* L., *Anacyclus clavatus* (Desf.) Pers., *Sonchus oleraceus* L. and *Lolium rigidum* Gaudin. All the phases of each crop sequence were present every year to take into account interannual variability. In the sub-plots, four levels of top-dressing N fertilization in cereals were assessed: 0, 40, 80 and 120 kg N ha^{-1}, considering the 80–120 kg N ha^{-1} range as the typical management practice for winter cereals in these rainfed cropping systems. Elementary plots were 3.5 ×12 m. Unless stated otherwise, the experimental year always refers to the harvest year.

2.2. *Cropping systems management*

The experimental field was located in a commercial farm managed under long-term no-till (>20 years). Crops were sown on November 4th 2019, October 27th 2020 and November 16th 2021 using a 3 m no-till drilling machine with disc openers (John Deere 1590, 19 cm row width). Wheat (cv. Filon, Florimond Desprez®) and barley (cv. Lagalia, LG seeds®) were sown at 370 seeds m^{-2}, pea (cv. Furious, LG Seeds ®) was sown at 100 seeds m^{-2} and faba bean (cv. Axel, Semences de France®) was sown at 40 seeds m^{-2}. Pre-emergence and post-emergence herbicides were applied according to weed pressure and expert advice to the cereals and grain legume crops. The MScc treatment was based on spontaneous species and did not receive herbicides. Each year, the spontaneous vegetation was terminated with a 3 m mulcher before weed seed set. Top-dressing N fertilisation was based on ammonium sulphate and applied to cereals according to each treatment (0, 40, 80 and 120 kg N ha^{-1}) on February 14th 2020, February 17th 2021 and February 23rd 2022. Legumes and MScc did not receive N fertilization. Harvest was performed with a plot combine harvester and grain yields are reported at 14% moisture content.

2.3. *Data acquisition and calculations*

Soil samplings were performed before sowing and after harvesting using a hydraulic helicoidal soil corer attached to an all-terrain vehicle. Soil samples were taken at two depth intervals, 0–30 and 30–60 cm. Soil nitrate (NO_3^-) was extracted with deionized pure water at a soil:water ratio of 1:5. The extracts were analysed with a continuous flow auto-analyzer (Multi-element analyser, Smartchem 200).

2.3.1. *Crop level – pea and faba bean*

Legume crops' aerial biomass was sampled twice over the growing season, at flowering and physiological maturity stages (BBCH 65 and BBCH 89, respectively (Lancashire et al., 1991)). Sampling areas consisted of 1 m along the sowing row for faba bean and 0.40 ×0.40 m squares for pea. Biomass N concentration was determined by dry combustion (model Truspec CN, LECO, St Joseph, MI, USA) of the whole aerial biomass at flowering and of grain and the rest of aerial crop biomass at physiological maturity. In the latter, grain N and non-grain N (referring to N in stems, leaves and empty pods) were summed up to calculate the total aboveground N acquisition. Legume N fixation was measured using the ^{15}N natural abundance method (Unkovich et al., 2008) (Eq. (1)). A dicotyledonous weed was used as a non-N-fixing reference plant and was collected in the MScc-0 N plots. Reference plants collected in each replication were used to calculate the legumes N fixation of each specific replication, thus minimizing spatial variability.

$$Ndfa = \frac{\delta^{15}N \quad reference \quad plant - \delta^{15}N \quad of \quad legume}{\delta^{15}N \quad reference \quad plant - B} x100 \quad (1)$$

Where Ndfa is N derived from atmosphere (N biologically fixed), $\delta^{15}N$ reference plant and $\delta^{15}N$ of legume are the parts per thousand deviations relative to the nominated international standard atmospheric N_2 (0.3663 atom% ^{15}N) for the reference plant and the legume crop, respectively. *B* value is a constant to account for the within-plant fractionation of ^{14}N and ^{15}N between shoots and nodulated roots (see Unkovich et al., 2008 for further explanation) and were −0.66 and −0.5 for pea and faba bean, respectively (Unkovich et al., 2008). Total N acquisition measured at each sampling date was separated between biologically fixed N (hereafter referred to as Ndfa) and N derived from soil (hereafter referred to as Ndfs).

2.3.2. Pre-crop level – effects on soil N mineralisation and wheat

Pre-crop effects were evaluated on the following wheat crop performance and soil N mineralisation. Soil N mineralisation was estimated annually through a balance of N uptake and soil mineral N variation (0–60 cm depth), following the methodology used by López-Bellido and López-Bellido (2001) and Plaza-Bonilla et al. (2021). These calculations were carried out using the wheat-0 N plots preceded by wheat, pea, faba bean and MScc to capture the effects of the alternatives on soil N mineralisation. As for the crop performance, a biomass sampling was carried out at physiological maturity (BBCH 92) and the following yield components were determined: spikes m^{-2}, number of grains $spike^{-1}$ and thousand-grain weight (TGW). The sampling area was 0.5 m along the sowing row. Biomass N concentration of grain and the rest of aerial crop biomass was determined by dry combustion (model Truspec CN, LECO, St Joseph, MI, USA). Grain N and non-grain N (the latter referring to N in stems, leaves and spike chaff) were summed up to calculate the total aboveground N uptake.

2.3.3. Cropping system level – productivity and N use efficiency

The cropping system's productivity was measured through energy and protein yields. Energy yields were calculated using the grain yield and the conversion factors 18.2 (wheat), 18.4 (barley), 18.3 (pea) and 18.7 (faba bean), extracted from www.feedipedia.com. For the protein yields, factors 5.49 (wheat), 5.45 (barley), 5.36 (pea) and 5.4 (Faba bean) were used for converting the N in the grain into crude protein (Mariotti et al., 2008). For each cropping system and N fertiliser rate, annualised energy and protein yields were calculated. Total N uptake (three experimental years) was also calculated for each treatment. N supply and its sources were calculated for each cropping system following Eq. (2).

$$N \quad supply = N_{fer} + N_{dfa} + N_{\min} \quad + \Delta SMN \quad (2)$$

Where N_{fer} is the amount of N fertiliser applied, N_{dfa} (N derived from atmosphere) is the amount of N fixed by the legume crops calculated using the natural abundance method, N_{min} is the N resulting from soil organic matter mineralisation, and ΔSMN is the variation in the soil nitrate content (0–60 cm depth) between sowing and harvest. In all cases, the sum of the three years was used.

Soil N mineralisation at the cropping system level was estimated using the balance described in the previous section in all 0 N barley and wheat phases. Grain legumes were not included in the mineralisation calculation as N fixation, though measured, is an external N input to the system, thus likely "underestimating" soil N mineralisation during the growing period of these crops (Plaza-Bonilla et al., 2021). Soil nitrogen mineralisation in the legume phase of the rotation was considered the same as in the cereal, as the previous crop (and more likely mineralizable N) was a cereal. Annual values for soil N mineralisation for each phase of the cropping systems were summed up to obtain the amount of mineralised N over the experimental period. Soil N mineralisation was assumed the same across the four N fertilizer rates.

Energy production to N tradeoff (ENT) and nitrogen use efficiency of protein production (NUEp) were calculated at the cropping system level following Eqs. (3) and (4).

$$ENT = \frac{energy \quad yield(GJ \quad ha^{-1})}{N \quad supply(kg \quad N \quad ha^{-1})} \quad (3)$$

$$NUEp = \frac{protein \quad yield(kg \quad ha^{-1})}{N \quad supply(kg \quad N \quad ha^{-1})} \quad (4)$$

Where *energy yield* refers to the sum of the three-year energy yields of each treatment, *protein yield* refers to the sum of the three-year protein yields of each treatment and *N supply* refers to the amount of N available in each cropping system calculated using Eq. (2).

2.4. Statistical analyses

Statistical analyses were carried out at the (i) legume crops level, (ii) pre-crop level and (iii) cropping system level using JMP 16Pro (SAS Institute Inc., 2019). At the legume crop level (i), separate analyses of variance (ANOVA) were performed for pea and faba bean with year as a single fixed factor. N fertilizer rate was not included in these analyses as no N fertilizer was applied to legume crops. The variables evaluated at this level were aboveground biomass at flowering, N sources (i.e., Ndfa and Ndfs) at flowering and physiological maturity, grain yield, grain N concentration, grain N and non-grain N. At the pre-crop level (ii), a mixed-model ANOVA for a split-split-plot design with three replications was used, with the year (main factor), pre-crop (secondary factor), N fertilizer rate (tertiary factor) and their interactions included as fixed factors in the ANOVA (Federer and King, 2008). At this level, soil N mineralisation, wheat grain yield, N uptake, grain protein concentration, spikes m^{-2}, grains $spike^{-1}$, and TGW were analysed. At the cropping system level (iii), a mixed-model ANOVA for a split–plot design with three replications was used, with the cropping system (main factor), N fertilizer rate (secondary factor) and their interaction included as fixed factors in the ANOVA. At this level, annual energy and protein yields, N uptake, ENT and NUEp were evaluated using the described model. At all levels, the block was included as a fixed effect in the model. At levels (ii) and (iii), N fertilizer rate was considered a continuous factor, whereas any other factor (year, pre-crop or cropping system depending on the level) was considered discrete. Whenever the N fertilizer rate was significant, linear regressions were fit between the N fertilizer rates (X-axis) and the variable (Y-axis). In case of a significant interaction including the N fertilizer rate, independent linear regressions were fit for each level of the discrete factor. Only significant regressions at $p<0.05$ are shown. The slopes of the different equations were compared using a pairwise comparison. For the discrete factors, HSD Tukey means separation test was performed for significant interactions and single effects.

3. Results

3.1. Climate conditions during the experimental period

Total precipitation and distribution differed between the three experimental years, with a total rainfall during the cropping season (November to June) of 422, 285 and 212 mm in 2019–2020, 2020–2021 and 2021–2022, respectively. The long-term average for the same period is 303 mm. No remarkable differences in the mean air temperature were recorded during the 2019–2020 and 2020–2021 experimental years compared to the average values for the area. During the third experimental year (2021–2022), a heat wave on 14–23 May 2022 was registered. Maximum temperatures were above 30ºC with 35–37ºC peaks (long-term average temperature for theisperiod was 18 ºC). Temperatures above the average continued from the 28th of May to the 20th of June, increasing the mean air temperature of May and June 2022 by 2.6 and 3.4 ºC, respectively (Fig. 1).

3.2. Crop level assessment: agronomic performance of pea and faba bean

Pea above-ground biomass at flowering was significantly higher in 2020 compared to 2021 and 2022 (Fig. 3A). Pea Ndfa followed the same trend as biomass (with 88 kg N ha^{-1} in 2020 and, on average, 24 kg ha^{-1} in 2021 and 2022), while Ndfs was not affected by the year with an average of 62 kg N ha^{-1} (Fig. 3A). Faba bean above-ground biomass significantly decreased throughout the experimental years (Fig. 3B). Similarly, faba bean Ndfa presented contrasting values of 212, 66, and 9 kg N ha^{-1} in 2020, 2021 and 2022, respectively (Fig. 3B). Faba bean Ndfs was higher in 2020 and 2021 (83 kg N ha^{-1} as an average) than in 2022 (29 kg N ha^{-1}) (Fig. 3B).

Pea could not be harvested for grain in 2020 due to severe lodging. Pea grain yield did not differ significantly ($p>0.2$) between the other years (2021 and 2022), with an average yield of 766 kg ha^{-1}. Pea grain N concentration was affected by the year ($p<0.01$), with 39 and 42 g kg^{-1} in 2021 and 2022, respectively, which represented a crude protein concentration in the grain of 21 and 22%. On average, 26% of pea N acquisition (27 kg N ha^{-1}) was removed as grain, without significant differences between 2021 and 2022 (Fig. 4A). However, in both years Ndfa was larger (35 kg N ha^{-1} on average) than grain N without differences between years (Fig. 4A). Non-grain N was larger in 2021 than in 2020 and 2022, and Ndfs followed the same trend (Fig. 4A). Faba bean yield was higher in 2020 (4251 kg ha^{-1}), while no differences were observed between 2021 and 2022 (1472 kg ha^{-1} on average). Grain N concentration was higher in 2022 than in 2021 (45 and 39 g kg^{-1}, respectively), and intermediate in 2020 (43 g kg^{-1}). On average, these values represented a grain protein concentration varying from 21% to 24%. Grain N presented contrasting values across the experimental seasons, with 158 kg N ha^{-1} removed in 2020 and an average of 52 kg N ha^{-1} in 2021 and 2022 (Fig. 4B). Non-grain N was not significantly different between the three experimental years (on average, 93 kg N ha^{-1}) (Fig. 4B). Faba bean nitrogen sources followed an opposite trend compared to pea. Nitrogen derived from atmosphere (Ndfa) was highest in 2020 with 200 kg N ha^{-1}, while an average of 52 kg ha^{-1} was observed in 2021 and 2022. Except in 2022, Ndfa exceeded grain N by 41 and 21 kg ha^{-1} in 2020 and 2021, respectively (Fig. 4B). On the other hand, the amount of Ndfs did not vary significantly throughout the experimental years (80 kg N ha^{-1}) (Fig. 4B).

3.3. Pre-crop level assessment: effects of different pre-crops on wheat

Soil N mineralisation was affected by the pre-crop x year interaction (Table 1). In 2021, pea as a pre-crop led to higher soil N mineralised compared to wheat with intermediate values for faba bean and MScc pre-crops (Fig. 5). Contrarily, no differences between pre-crops on soil N mineralisation were observed in 2022, with a trend of higher values under pea and faba bean (on average, 94 kg N ha^{-1}) compared to wheat and MScc pre-crops (on average, 45 kg N ha^{-1}) (Fig. 5).

Wheat grain yield was affected by the pre-crop x year interaction (Table 1). In 2021, legumes and MScc pre-crops led to significantly higher yields (4986 kg ha^{-1}, on average) than continuous wheat (4121 kg ha^{-1}). In 2022, the differences between pre-crops were not significant, although a trend to slightly higher yields was observed with legume pre-crops (2637 kg ha^{-1}, on average) compared to wheat and MScc pre-crops (2182 kg ha^{-1}, on average) (Fig. 6).

Wheat N uptake was affected by the single effects of year, pre-crop and N fertilizer rate (Table 1). Wheat N uptake was 60% higher in 2021 than in 2022 (Fig. 7A) and overall, it was higher when faba bean instead of wheat was the pre-crop, with pea and MScc pre-crops showing intermediate values (Fig. 7B). On average, in 2021 and 2022, wheat N uptake responded positively to N fertilization, increasing from 111 to 160 kg ha^{-1} as the N fertilizer rate increased from 0 to 120 kg N ha^{-1} (Fig. 7C). Wheat grain protein content was significantly affected by the pre-crop x N fertilizer rate x year interaction (Table 1). In 2021, overall lower grain protein contents were observed, and only wheat preceded by faba bean responded significantly to N fertilization (Fig. 8). Although not significant, wheat pre-crop led to lower grain protein concentration in wheat regardless of the N fertilizer rate. In 2022, two types of responses to N fertilization were observed. Legume pre-crops presented a higher, but constant, grain protein content across N fertilizer rates (144 g kg^{-1}, on average). Meanwhile, wheat and MScc pre-crop showed a positive and significant response to N fertilization rate (Fig. 8).

Wheat spikes m^{-2} were affected by the pre-crop x nitrogen interaction and the year as a single effect (Table 1). A positive response spikes m^{-2} to increasing N fertilizer rates in the wheat pre-crop was observed, with 1.66 additional spikes per each kg of N ha^{-1} applied (Fig. 9A). Contrarily, wheat spikes m^{-2} did not respond to N fertilization when the pre-crops were pea, faba bean and MScc, presenting average values of 529, 541 and 501 spikes m^{-2}, respectively, as an average of 2021 and 2022 (Fig. 9B, C, D). Regarding the year single effect, wheat spikes m^{-2} were greater in 2021 than in 2022 (583 and 447 spikes m^{-2}, on average for all treatments). Wheat grains spike^{-1} were affected by the year effect

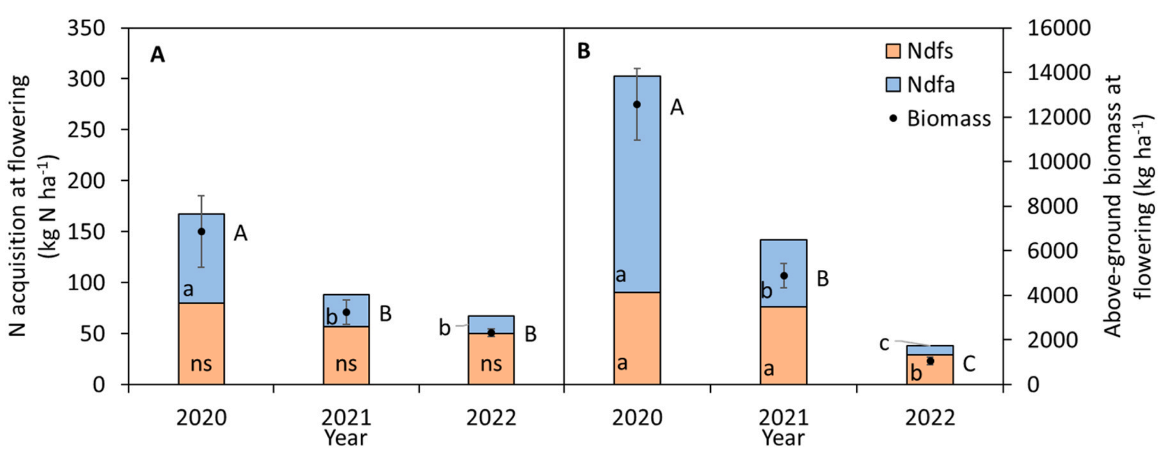

Fig. 3. Above-ground biomass (dot series), nitrogen derived from atmosphere (Ndfa) and nitrogen derived from soil (Ndfs) for pea (A) and faba bean (B) across the three experimental years. Within each subfigure, uppercase and lowercase letters indicate significant differences between years at $p<0.05$ for the biomass and N source (Ndfa and Ndfs) values, respectively. Error bars refer to standard error.

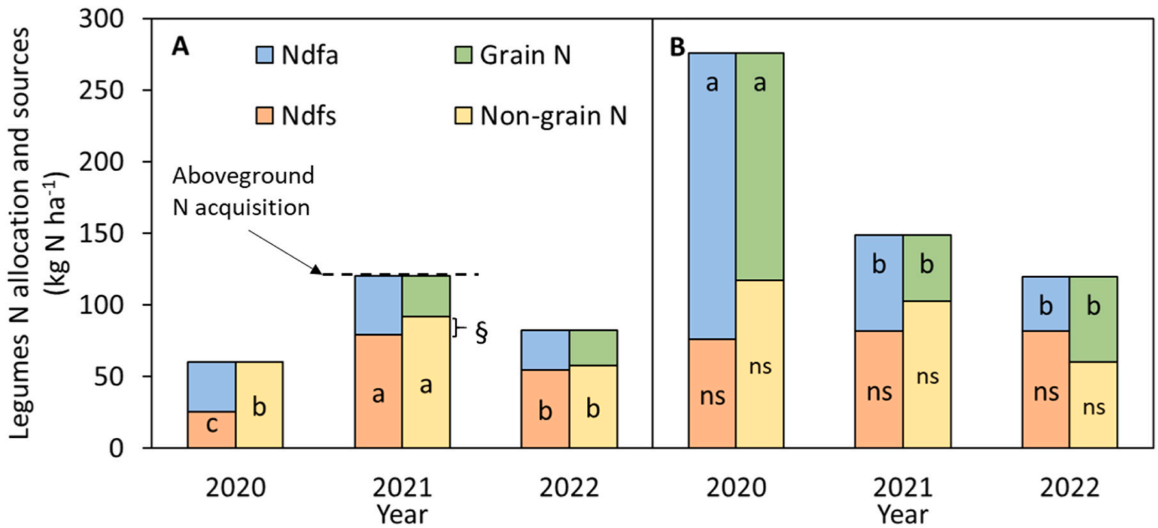

Fig. 4. Pea (A) and faba bean (B) biomass N allocation (grain N and non-grain N) and sources (nitrogen derived from atmosphere (Ndfa) and nitrogen derived from soil (Ndfs)) across the three experimental years. Within each subfigure and variable, levels not connected by the same letter are significantly different at p<0.05. ns: not significant. §: net N balance (N fixed minus N exported).

Table 1
Effects (P-values) of year (Y), pre-crop (PC), nitrogen fertilizer rate (N) and their interactions for soil N mineralisation (N min), wheat grain yield, N uptake, grain protein concentration, spikes m^{-2}, grains $spike^{-1}$ and thousand-grain weight (TGW). **Bold** p-values indicate p<0.05. na: not applicable.

Factor	Soil variable	Wheat variables					
	N min	Grain yield	N uptake	Grain protein	Spikes m^{-2}	Grains $spike^{-1}$	TGW
Year (Y)	**0.015**	**0.018**	**0.035**	**0.002**	**0.018**	**0.005**	**0.003**
Pre-crop (PC)	**0.009**	**0.001**	**0.019**	**<0.001**	0.627	0.811	0.404
PC x Y	**0.031**	**0.024**	0.130	0.150	0.098	0.703	0.358
Nitrogen (N)	na	0.157	**<0.001**	**<0.001**	0.059	1.000	**<0.001**
N x Y	na	0.556	0.953	0.285	0.443	0.098	**0.029**
PC x N	na	0.347	0.119	**0.003**	**0.042**	0.541	0.969
PC x N x Y	na	0.237	0.245	**0.035**	0.462	0.632	0.075

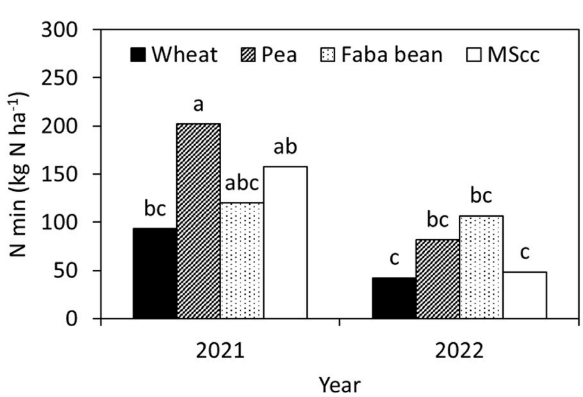

Fig. 5. Soil N mineralisation (N min) during the wheat growing period depending on the pre-crop x year interaction. Levels not connected by the same letter are significantly different at p<0.05.

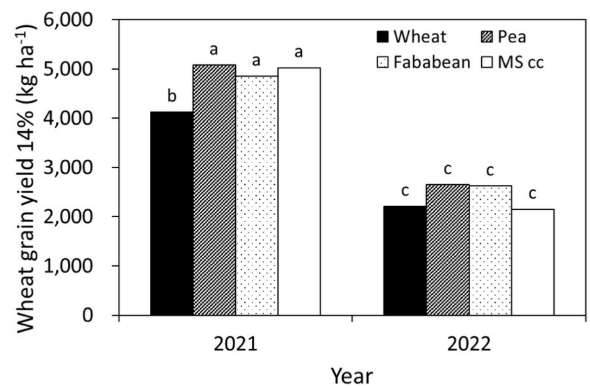

Fig. 6. Wheat grain yield (14% moisture) depending on the pre-crop x year interaction. Levels not connected by the same letter are significantly different at p<0.05.

(Table 1), with 36.1 and 31.1 grains $spike^{-1}$ in 2021 and 2022, respectively. Thousand-grain weight was affected by the nitrogen x year interaction (Table 1). In 2021, a reduction in TGW as the N fertilizer rate increased was observed, while in 2022 no response to N fertilization was detected, with an average TGW of 25.1 g (Fig. 9E).

3.4. Cropping systems performance

Energy yield was only affected by the cropping system (Table 2), with the WCS (81 GJ ha^{-1} yr^{-1}) presenting higher yields than the FCS (61 GJ ha^{-1} yr^{-1}). The PCS and the MSCS presented lower energy yields than the previous ones, averaging 50 GJ ha^{-1} yr^{-1}. Protein yield was affected by the cropping system x nitrogen interaction (Table 2). The four cropping systems responded positively to increasing levels of N fertilization (Fig. 10). For instance, protein yield varied from 212 to 464 kg protein ha^{-1} in the WCS while in the MSCS the variation was from 178 to 286 kg protein ha^{-1} across the different N fertilizer rates (Fig. 10). Taking WCS as a reference, PCS and MSCS presented a lower

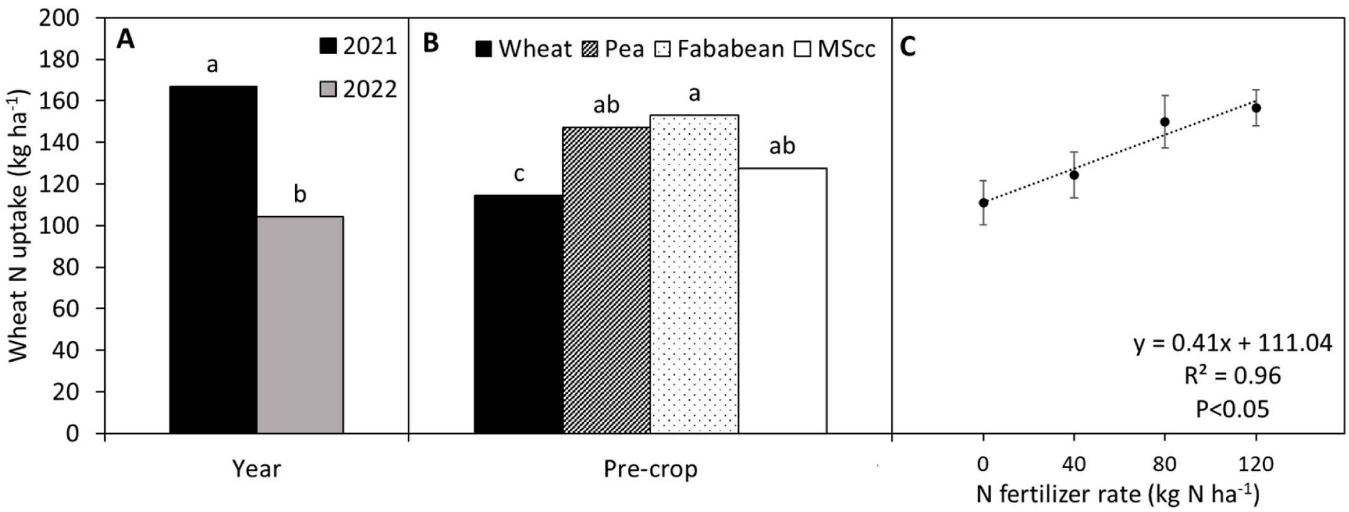

Fig. 7. Wheat N uptake depending on the year (A), pre-crop (B) and N fertilizer rate (c). Within each sub-figure, levels not connected by the same letter are significantly different at p<0.05. Error bars refer to standard error.

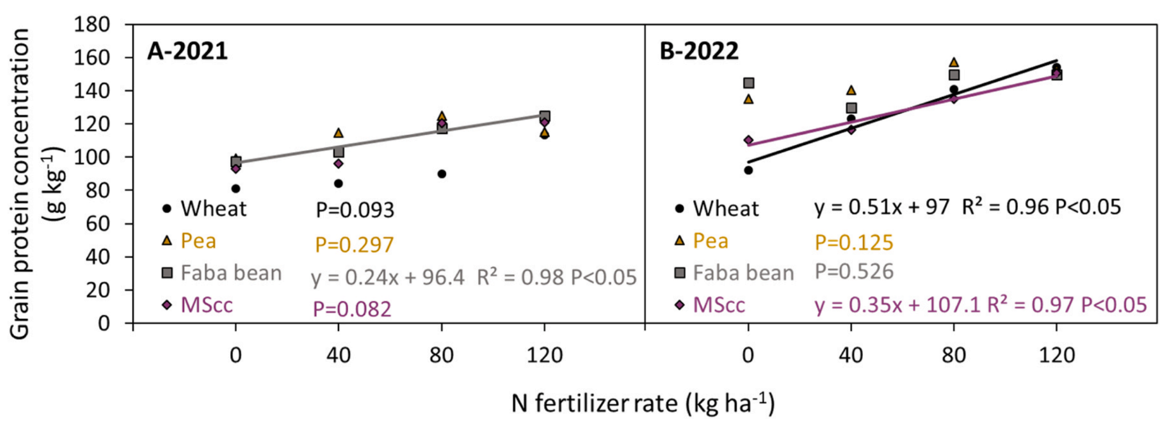

Fig. 8. Wheat grain protein concentration (g kg^{-1}) in 2021 (A) and 2022 (B) depending on the pre-crop x N fertilizer rate x Year interaction.

response to N fertilizer. The FCS cropping system did not present significant differences in the regression slope with any of the other cropping systems. Higher protein yields across all N fertilizer rates were found in FCS, and below 40 kg N ha^{-1} in PCS, when compared with WCS and MSCS.

N acquisition and N supply response to N fertilization in the three experimental years (values summed up) were plotted for each cropping system (Fig. 11). Soil nitrate content variation was stable across cropping systems and N fertilizer rates with an average value of 80–100 kg N ha^{-1}. Soil N mineralisation (N min) was on average 57, 38 and 31 kg ha^{-1} higher in the PCS, FCS and MSCS, respectively, compared to the WCS and assumed constant throughout the four N fertilizer rates (see Sections 2.3 and 3.3). Biological N fixation (Ndfa) was only present in PCS and FCS (as they included legume crops) with the latter presenting larger values, following the trends described for pea and faba bean crop level results (Section 3.1). No effect of N fertilizer rate in Ndfa was found (p>0.7 for faba bean and p>0.5 for pea). Finally, N fertilization varied accordingly to the experimental treatments imposed, with higher values in the WCS as N fertilization was applied in all the phases of the cropping system.

Crops N acquisition was impacted by the cropping system x N fertilizer rate interaction (Table 2). In the WCS, PCS and FCS, N acquisition linearly increased with N fertilizer rates, while in the MSCS no significant linear response to N fertilization was detected (on average, 293 kg N ha^{-1}) (Fig. 11). Wheat cropping system (WCS) presented the greatest slope, being different from PCS but not from FCS. Overall, the highest N acquisition levels were observed in the FCS (404 and 553 kg N ha^{-1} in 0 and 120 kg N ha^{-1}, respectively). The WCS presented the smallest differences between N acquisition and N supply (Fig. 11 WCS), which led to the highest ENT. For this indicator, WCS had the highest negative response to N fertilizer rates with values ranging from 0.71 to 0.43 GJ kg N $supply^{-1}$ for 0 and 120 kg N ha^{-1} fertilization, respectively. The PCS, FCS and MSCS also presented a negative response to increasing N fertilizer rates (not significantly different among them) with average values of 0.32 and 0.22 GJ kg N $supply^{-1}$ in the 0 and 120 kg N ha^{-1}, respectively (Fig. 11). Nitrogen use efficiency for protein production (NUEp) was not affected by any factor (Table 2), presenting an average value of 1.69 kg protein kg N $supply^{-1}$ across cropping systems and N fertilizer rates.

4. Discussion

4.1. Cropping systems productivity and efficiency

This study aimed to assess crop diversification and N fertilization as strategies for more sustainable use of N in long-term no-till Mediterranean rainfed systems. In that regard, a cropping system-level study is necessary for a holistic assessment of the alternatives (Reckling et al., 2016). Along with diversification, a reduction in energy yield was observed, a phenomenon already reported by Notz et al. (2023) across

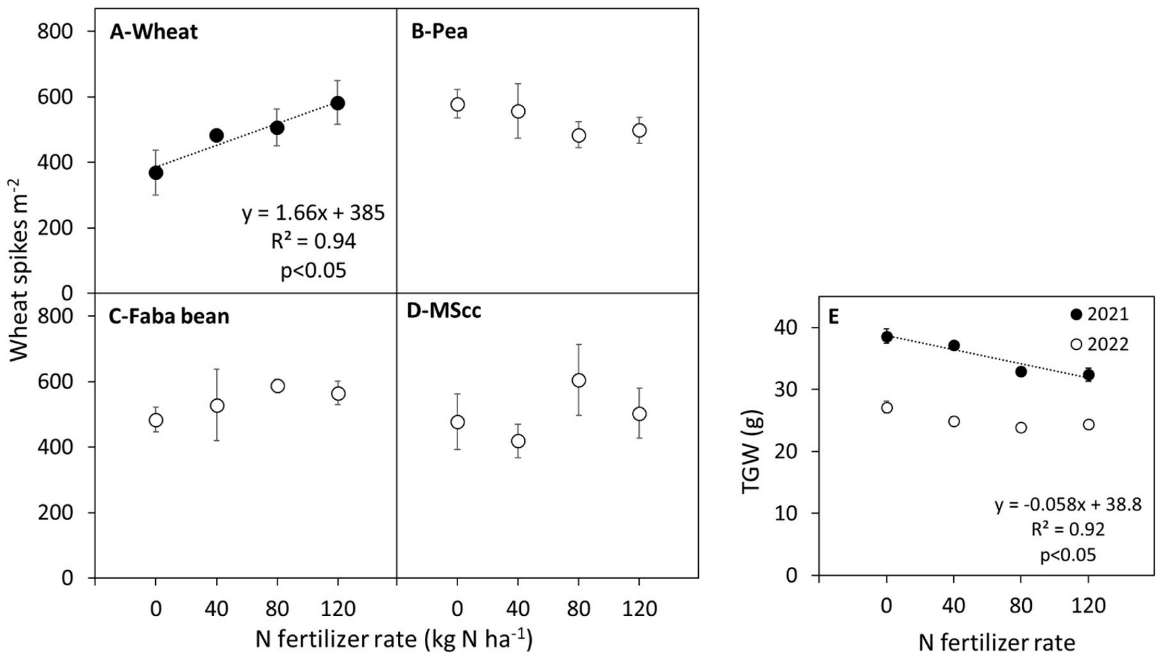

Fig. 9. Wheat spikes m^{-2} depending on the pre-crop x N fertilizer rate interaction (subfigures A, B, C and D) and thousand-grain weight (TGW) depending on the year x N fertilizer rate interaction (subfigure E). In all cases, full dots indicate a significant regression and blank dots indicate non-significant regression thus no equation is presented. Error bars refer to standard error.

Table 2
Effects (P-values) of cropping system, N fertilizer rate and their interaction for energy and protein yields, N uptake, and N use efficiency of energy production (NUEe) and protein production (NUEp). **Bold** p-values indicate $p<0.05$.

Factor	Energy yield	Protein yield	N uptake	NUEe	NUEp
Cropping system (CS)	**0.001**	**0.028**	**0.001**	**0.001**	0.096
Nitrogen (N)	0.291	**0.001**	**0.001**	**0.001**	0.494
CS x N	0.363	**0.014**	**0.001**	**0.001**	0.317

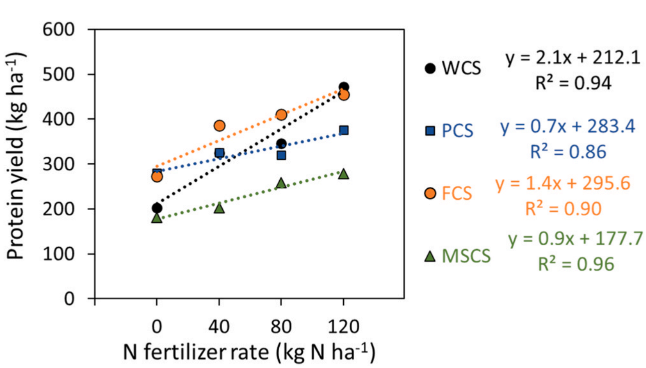

Fig. 10. Linear regression between increasing levels of N fertilization and annualised protein yield of four cropping systems (wheat cropping system: WCS; pea cropping system: PCS; faba bean cropping system: FCS; multiservice cover crop cropping system: MSCS).

different cropping systems in Europe. We did not observe a clear response to N fertilization in energy production, probably due to high soil mineral nitrogen contents in 2020 (when the experiment began) and overall low productivity in 2021 and 2022. The lack of response to N fertilization, a common feature under Mediterranean rainfed conditions (Ryan et al., 2009), impacted the ENT with a clear reduction as N supply increased due to increasing N derived from N fertilization. In cereal-based cropping systems under similar environments, reductions in NUE (kg grain kg N supply^{-1}) are reported, however at the crop level. For instance, a decrease from 22 to 11 kg wheat grain kg N^{-1} where recorded in S Spain (López-Bellido and López-Bellido, 2001) after increasing the N fertilizer rate from 0 to 150 kg N ha^{-1} As well, the non-response to N fertilization would also increase the cropping system C footprint thus increasing the environmental cost of this particular agricultural production (Liu et al., 2016).

Crop diversification reduced ENT overall, e.g. 63% reduction in the FCS compared to WCS at 0 N. These results contrast with some existing literature under Mediterranean rainfed conditions, where positive effects of crop rotation on the following wheat NUE are reported (Espinoza et al., 2015; López-Bellido and López-Bellido, 2001; Souissi et al., 2020). The discrepancy between the cited studies and ours is explained by the boundaries in the ENT and NUE calculations (either for grain or energy production). While the cited studies focus on the pre-crop level, including only the following wheat in the NUE calculation and excluding the legume phase, we show that upscaling the boundary to the cropping system level leads to an ENT decrease in systems with alternative crops. Such a decrease is caused by the energy productivity reduction and the increased N supply through biological N fixation. In that regard, legume crops invest part of their photosynthetic energy to biological N fixation, resulting in lower energy output at harvest. Simultaneously, they act as a N source to the cropping systems, which has to be accounted for in the ENT or NUEp calculations (EU Nitrogen Expert Panel, 2015).

While the effect of N fertilization on grain (and energy) yield was negligible, increasing N fertilizer rate led to higher protein yields at the cropping system level. Protein increases rather than grain yield increases are a typical response to N fertilization under Mediterranean conditions (Savin et al., 2019). However, in this study, the importance of this effect depended on the cropping system. For instance, protein yield presented a smaller response to N fertilization in the FCS compared to WCS. These results highlight the importance of legume crops as a sustainable strategy for protein production increase in low-input cropping systems. Similarly, Costa et al. (2021) estimated an increase in crude protein production (from 202 to 320 kg ha^{-1} yr^{-1}), when diversifying a cereal-based rotation with faba bean in Calabria, southern Italy.

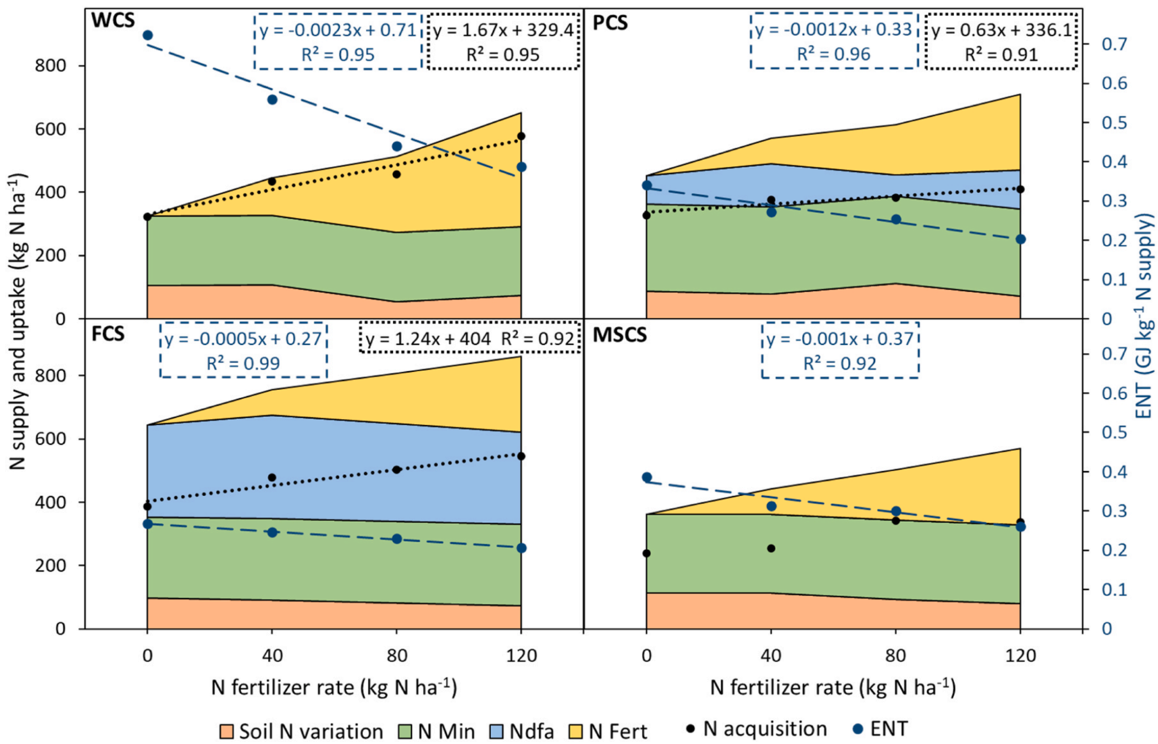

Fig. 11. N supply sources (soil N variation, difference between soil nitrate at sowing and harvest; N min: soil N mineralisation; Ndfa: N derived from atmosphere; N fert: fertilizer N), N acquisition (black dots, lines and equations), and energy production to N tradeoff (ENT, blue dots, lines and equations) of four cropping systems (wheat cropping system (A-WCS), pea cropping system (B-PCS), faba bean cropping system (C-FCS) and multi-service cover crop cropping system (D-MSCS)) combined with increasing rates of N fertilizer.

Contrary to the case of the ENT, protein productivity increased proportionally to the higher availability of N, either from the fertilizer (effect of the N fertilization) or the biological N fixation (effect of the legume introduction) (Fig. 10), leading to a stable NUEp.

4.2. Soil N mineralisation and wheat performance affected by the pre-crops

Soil N mineralisation was estimated using a simplified balance assuming negligible N losses, as proposed by Angás et al. (2006) for a similar area and soils. In our case, the average values under wheat pre-crop are similar to previous studies carried out in the area in cereal mono-crops. For instance, (Plaza-Bonilla et al., 2021, 2017b) reported a soil N mineralisation of 41–51 kg N ha^{-1} in a similar environment under no-till. The cited studies, however, referred to continuous cereal cropping systems. In our case, pre-crops had a positive impact on N mineralisation, increasing soil mineral N availability for the following crops in a sustainable way (Guinet et al., 2020). On average, alternative pre-crops increased mineralized soil N by 51 kg ha^{-1}. Similar results were reported by McBeath et al. (2015) in a semi-arid Mediterranean area in Australia, with increases of 37–47 kg N ha^{-1}.

Pre-crop effects are separated into N-related effects, directly linked to the provision of N, and the break crop effect, related to the reduced risk of weeds, pests and diseases, soil quality, etc. (Chalk, 1998; Notz and Reckling, 2022). Alternative Pre-crops increased succeeding wheat grain by 264–844 kg ha^{-1}, on average, representing a relative increase of 11–21%. These findings are in line with the range of pre-crop benefits on cereals for European Mediterranean areas of 240–960 kg grain ha^{-1} (23% yield increase in proportion) established by Preissel et al. (2015). Contrary to what was found by other authors (e.g. Kirkegaard et al., 2008; Preissel et al., 2015), pre-crop benefits were not affected by the N fertilizer rate –overall, cereal yields in this experiment responded mildly to N fertilization. Wheat N uptake followed a similar trend to grain yields concerning the pre-crop effect and also showed a positive correlation with increasing N fertilizer rates. This phenomenon is a common feature under Mediterranean conditions (Savin et al., 2019), as above-ground N uptake is usually less restricted than resource allocation to the grain (yield) because it occurs under lower temperatures and, usually, greater water availability (Barraclough et al., 2014). The alternative pre-crop effects also increased wheat grain protein content (Fig. 8), but with a different response each year. In 2021, the increase was independent of N fertilization, suggesting that the break crop effect could have been the primary driver. Instead, in 2022, wheat and MScc pre-crops led to similar grain protein contents when high N rates were applied, indicating a positive N effect of the legume crops on the following wheat.

When splitting the yield into its components we observed that the pre-crop affected only the number of spikes m^{-2}. The average spikes m^{-2} after pea, faba bean and MScc were equivalent to the spikes in wheat pre-crop when applying, on average, 83 kg N ha^{-1}, respectively. The lack of a response after an alternative pre-crop indicates that there was a N-related pre-crop effect, as spikes m^{-2} determination takes place at the tillering stage and it is influenced by N availability (Ryan et al., 2009). This hypothesis is also supported by the increased soil N mineralisation observed after the grain legumes and MScc, increasing the wheat N supply during a period of water availability and mild temperatures (i.e. winter). On the contrary, grains $spike^{-1}$ and TGW yield components varied with the year, reflecting the contrasting conditions of the 2021 and 2022 seasons, with the latter being dryer and affected by a severe heat wave during the grain-filling period. The hot and dry conditions during cereals' reproductive stages are a constraining factor for yield determination under Mediterranean conditions, hindering the expression of any pre-crop effect on the grains $spike^{-1}$ and TGW (Savin et al., 2015).

4.3. Agronomic performance of grain legumes

Across the three experimental years, pea biomass at flowering was largely influenced by the precipitation received, with larger biomass production in the wettest year (2020) and lower in the dryer ones (2021 and 2022). The variation in biomass accumulation affected biological N fixation, which ranged from 24% to 54%, (17–88 kg N ha^{-1}). These results are in agreement with Oliveira et al. (2019) in Portugal (humid Mediterranean climate, precipitation of 1023 mm annually and sandy soils) of 54% of Ndfa and 38 kg N ha^{-1}. Pea yield fell below (< 1000 kg ha^{-1}) the average values reported for other Mediterranean areas, where Siddique et al. (1999) established a range of 1000–2300 kg ha^{-1} across Australia. The low yields reported in 2021 and 2022 were a consequence of scarce precipitations (and high temperatures during grain filling in 2022). The first experimental year (2020) presented potentially adequate conditions for pea production, also measured at the flowering stage with the highest biomass accumulation across the experimental period. However, a disease complex and subsequent lodging caused a complete loss of production. This phenomenon has been widely reported by several authors as a major cause of pea yield penalties (Gaulin et al., 2007; Le May et al., 2009; Oliveira et al., 2019),and can limit the farmer's adoption of such crops. In that regard, pea-cereal intercropping could be an alternative to help prevent lodging (Podgórska-Lesiak and Sobkowicz, 2013).

Faba bean showed a higher reliance on biological N fixation compared to pea, with the proportion of Ndfa ranging from 22% to 74% (a range of 9–212 kg N ha^{-1} at flowering, Fig. 3B). We observed that the proportion of Ndfa was highest in the wettest year and lowest in the driest, in agreement with Ma et al. (2022), who identified soil moisture as one out of the three (along with soil temperature and soil mineral N concentration) most important drivers of biological N fixation globally through a modelling approach using LPJ-GUESS model. Opposite to Ma et al. (2022), in our study, the highest N fixation rates (2020) coincided with the highest soil nitrate content (ca. 140 kg N ha^{-1}, 0–60 cm, pre-sowing), highlighting that soil moisture might be more limiting than soil mineral N concentration under Mediterranean rainfed conditions. In this context, Ruisi et al. (2017) evaluated 25 years of seven field experiments including faba bean across Italy and showed positive correlations between Ndfa amount and biomass accumulation, grain yield and N uptake. Qualitatively comparing our data with Ruisi et al.'s correlation we observe that our results agree with theirs in the trend (similar slopes) but with a lower intercept in all cases. Fababean yield was affected by year, with 4250 kg ha^{-1} in 2020 due to the high rainfall in that season. Instead, in 2021 and 2022, yield levels were much closer to those under Mediterranean conditions, falling in the 1000–2100 kg ha^{-1} range reported by Siddique et al. (1999). As oposed to pea, faba bea presented grain yields closer to the ones reported for similar environments. While currently pea is the preferred grain legume crop for farmers of the area, our results show that faba bean can be an alternative in terms of yield and N fixation.

Net N balance for both legumes (amount of grain N vs. Ndfa (Chalk, 1998)) was close to neutral for pea (when harvested) with values around 8 kg N ha^{-1}, while in faba bean it ranged from 41 to −21 kg N ha^{-1}, and was proportional to the accumulated biomass. Legumes net N balance has been widely discussed in the literature with positive, negative and neutral values across species and cropping systems (Evans et al., 2001; Rochester et al., 1998). Our results, within the reported ranges, also present a large variation, emphasizing the site- and year-specific nature of the net N balance of legumes. Nonetheless, grain legumes provide high-quality (low C:N and high N:lignin ratios) crop residues that are more easily decomposable compared to cereals (McDaniel et al., 2014).

5. Conclusions

We conclude that a multiscale approach (crop, pre-crop and cropping system level) is required to evaluate crop diversification and N fertilization to identify levers towards sustainable and N efficient cropping systems for long-term no-till Mediterranean rainfed conditions. Under the studied conditions, growing legume crops represented a net input of N to the cropping system (biologically fixed N was larger than N removed by grain) in most cases. Although pea is more commonly grown in the area and preferred by feed producers, faba bean outyielded pea in all agronomic indicators. Introducing a grain legume or a MScc had a positive effect on the amount of soil N mineralised during the following wheat crop, which translated into increased resources for tillering and spikes determination, which finally improved wheat yield. Our results contribute to achieving low-input cropping systems, especially in the case of synthetic N. At the cropping system level, crop rotation and N fertilization had contrasting effects. Energy productivity was reduced when cropping systems were diversified, without a significant response to N fertilization. Instead, protein production increased significantly when cropping systems were diversified with legumes, with a differential response to N fertilization depending on the legume crop. While at low N fertilizer rates both systems including legumes (PCS and FCS) produced more protein than WCS, FCS outyielded WCS regardless of the N fertilizer rate. We conclude that crop diversification and N fertilizer adjustment are viable strategies to increase plant protein productivity sustainably in long-term no-till Mediterranean rainfed cropping systems.

CRediT authorship contribution statement

Plaza-Bonilla Daniel: Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Simon-Miquel Genís:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Reckling Moritz:** Writing – review & editing, Supervision, Formal analysis.

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.

Data availability

Data will be made available on request.

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