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Cereal and oil seed crops response to organic nitrogen when grown in rotation with annual aerial-seeded pasture legumes

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Abstract

Nitrogen fixation from pasture legumes is a fundamental process that contributes to the profitability and sustainability of dryland agricultural systems. The aim of this research was to determine whether well-managed pastures, based on aerial-seeding pasture legumes, could partially or wholly meet the nitrogen (N) requirements of subsequent grain crops in an annual rotation. Fifteen experiments were conducted in Western Australia with wheat, barley or canola crops grown in a rotation that included the pasture legume species French serradella (*Ornithopus sativus*), biserrula (*Biserrula pelecinus*), bladder clover (*Trifolium spumosum*), annual medics (*Medicago* spp.) and the non-aerial seeded subterranean clover (*Trifolium subterraneum*). After the pasture phase, five rates of inorganic N fertilizer (Urea, applied at 0, 23, 46, 69 and 92 kg/ha) were applied to subsequent cereal and oil seed crops. The yields of wheat grown after serradella, biserrula and bladder clover, without the use of applied N fertilizer, were consistent with the target yields for growing conditions of the trials (2.3 to 5.4 t/ha). Crop yields after phases of these pasture legume species were similar or higher than those following subterranean clover or annual medics. The results of this study suggest a single season of a legume-dominant pasture may provide sufficient organic N in the soil to grow at least one crop, without the need for inorganic N fertilizer application. This has implications for reducing inorganic N requirements and the carbon footprint of cropping in dryland agricultural systems.

Introduction

Australian farmers grow legumes in rotation with crops to help manage weeds, pests and diseases in their production systems (Angus and Peoples, 2012). A significant advantage of legumes in these systems is related to their ability to form a mutually beneficial symbiotic association with root nodule bacteria and convert atmospheric nitrogen (N_2) into ammonia before oxidizing to become useful for plants (Armstrong *et al.*, 1997 and Sprent and Parson, 2000, Berry *et al.*, 2002, Howieson *et al.*, 2008). Modern Australian agricultural systems have relied upon N_2-fixation from rhizobia-legume symbioses since European colonization in the late 18th century (Donald 1960; Howieson *et al.*, 2008). This N_2-fixation is currently estimated to be worth more than $2 billion per annum (Herridge *et al.*, 2008).

A recent review by Angus *et al.* (2015) highlighted that in over 70 studies, wheat grown in the season after a break crop, especially after grain legumes (harvested), yielded significantly more than wheat that was sown after wheat. Similar results were also reported by Seymour *et al.* (2013), who evaluated over 167 experiments conducted in the Western Australian wheatbelt between 1974 and 2007. The results demonstrated that cereal crops, following legume break crops, usually yield higher than continuous cereal.

Pasture legumes are an important component of the grazing systems and are implemented worldwide as a break crop in mixed farming systems, and they remain a dominant feature of dryland farming systems in sustaining the fertility of the soils (Kirkegaard *et al.*, 2011).

Grazing systems are much more diversified, since they can range from extensive systems with low stocking densities, long grazing periods and low use of concentrates to intensive systems with high stocking densities (Meul *et al.*, 2012). Grazing has been found to be associated with lower production costs (Soriano *et al.*, 2001), lower energy and mineral fertilizer use.

However, quantifying the benefits to crops from a grazing pasture legumes system is more complicated than for grain or pulse legumes, as pasture legumes are grazed by animals, and benefits are confounded by factors such as nutrient cycling and the removal of N during grazing or forage removal as hay and silage (Unkovich *et al.*, 1997).

All herbivores return N through faeces and urine, enhancing N cycling (Hobbs 1996; Frank *et al.*, 2000). However, the scale of redistribution of N is different for different herbivore sizes: large herbivores deposit faeces in large amounts in few patches, whereas smaller herbivores produce small pellets that are widely distributed. N from small herbivore faeces may be more available for plants (Bakker *et al.*, 2004).

Nonetheless, the role of legume-based pastures and their effect on total soil N in the dryland cereal cropping areas of southern Australia, West Asia, North Africa and South America have been outlined by Rowland *et al.* (1988), Ates *et al.* (2014), Zuhair and Ryan (2006) and Fischer *et al.* (2002). These researchers concluded that cereal/pasture legume rotations, in addition to being biologically and economically attractive, also improved soil fertility and thus promoted sustainability in fragile semi-arid areas. However, the amount of nitrogen (N) fixed by pasture legumes (which excludes that taken up from soil reserves), is still poorly documented compared with those of the grain legumes or perennial legumes (Stopes *et al.*, 1996, Loges *et al.*, 1999). Using the ^{15}N natural abundance technique, Peoples *et al.* (1998) estimated that the N fixed by subterranean clover-based pasture was between 5 and 238 kg/ha, and they suggested that 20–25 kg of N is fixed for every 1 t of dry matter produced.

Since these estimates, there has been considerable success in domesticating new species of pasture legumes that are adapted to the challenging, acidic and sandy soils in Western Australia (Howieson *et al.*, 2000). This has been enabled through co-development of adapted and persistent root nodule rhizobial strains (Howieson and Ballard, 2004). From these research programmes several novel genera/species/cultivars have been domesticated and commercialized globally. This includes French serradellas (*Ornithopus sativus* Brot.), yellow serradellas (*Ornithopus compressus* L.), biserrula (*Biserrula pelecinus* L.), gland clover (*Trifolium glanduliferum* Boiss.), bladder clover (*Trifolium spumosum* L.) and eastern star clover (*Trifolium dasyurum L.*) (Howieson *et al.*, 2000, Loi *et al.*, 2001, 2005, 2014, Nutt *et al.*, 2021*a*). These species have also been selected for their deep root systems (which extend the growing period), seed dormancy mechanisms that protect seeds from germinating during out-of-season rainfall and allow regeneration after a cropping phase (Loi *et al.*, 2005; Nutt *et al.*, 2021*a*), tolerance to pests and diseases and ability to harvest large quantities of seed with non-specialist machinery (Howieson *et al.*, 2000; Loi *et al.*, 2005, 2008, 2010 and 2012). While these newer species are being steadily adopted by farmers due to their superior productivity and nutritional value, as well as benefits associated with a break crop, there has been little quantification of the N fixation benefits.

In a major advancement from conventional practice, growers are now summer-sowing (rather than early-winter sowing) unprocessed, un-scarified and/or stored seed of some of these species. This practice alleviates competition for limited labour and machinery at the time cereal crops are sown. The high and fluctuating summer and autumn temperatures enable natural hard-seed breakdown (loss of seed dormancy), reducing a significant processing cost prior to pasture establishment (Harrison *et al.*, 2021, Howieson *et al.*, 2021, Nutt *et al.*, 2021*a*). This practice has the additional benefit of early and vigorous seedling establishment and can lead to an increase in pasture legume biomass production in early winter when low biomass is a major constraint to livestock production. If these systems result in more biomass production over the growing season, it is reasonable to anticipate a concomitant increase in N-fixation. The new approach to pasture establishment, called ‘Summer sowing’ (Nutt *et al.*, 2021*b*), has yet to be quantified for N-fixation, particularly in relation to the net benefit of this in rotation with cereals.

The aim of this work was to measure crop yields following one year of the newly commercialized annual legume pasture species (serradella, biserrula and bladder clover). We tested the hypothesis that for different pasture legume species, a one-year pasture phase could deliver sufficient organic (fixed) N to produce equivalent grain yields to fertilizer N in a following crop. Further, we studied the N requirement of the crop sequence for up to three years following the pasture legume to determine the legacy of the fixed N relative to rates of fertilizer N.

Material and methods

A series of experiments were conducted over several years across different locations of the Western Australian wheat-belt (Table 1). The first two experiments (Legume *v.* Fallow) compared the effect of pasture legumes or a chemical fallow on the yield of the following crop. The second set of experiments (multiple-year response) measured crop responses in the first, second and third crops after a single year of pasture. The third set of experiments (single-year response) measured yield and N response in cereals grown after one year of different pasture legumes. All sites were soil sampled in autumn before crop establishment to create a base line.

Legume v. *fallow*

Experiments 1 and 2 compared a wheat or canola crop following one year of either French serradella or a chemical fallow. In the crop year, the experimental area was supplied with either four or five N rates (see below), applied as strip-plots and replicated three times.

Experiment 1. Esperance

Pasture phase

Experiment 1 was located near Esperance in south-eastern Western Australia (33°33′51.58″S, 121°44′14.964″E) in a paddock where the pasture legume French serradella Cadiz (soft seeded) was grown in 2013. During the pasture phase in 2013, the site was sown with 15 kg/ha of Cadiz pods at 1 cm depth, using no-till machinery with knife points followed by press wheels at a row spacing of 25 cm. Within the site, a strip of 12 × 100 m was left unsown, and the weeds were controlled with a non-selective herbicide during the year to simulate a chemical fallow. The site was fertilized with 100 kg/ha of superphosphate (9, 11 kg S/ha) and 35 kg/ha muriate of potash (18 kg K/ha). The pasture was allowed to grow all year deferring the grazing to late October. Total above-ground biomass was collected from the serradella plots at the time of peak biomass on the 19th of September 2013. Biomass samples were collected by cutting at ground level (with knives) from four randomly placed open quadrats (0.1 m^2).

Crop phase

At the break of season, in May 2014, a wheat crop (Mace) was sown by the farmer at a rate of 80 kg/ha of seed, with row spacing of 25 cm, after the site was sprayed with glyphosate 1 L/ha (540 g ai/l) to kill weeds. During the season the farmer followed standard practices for crop management (Anderson and Garlinge, 2000). N fertilizer was applied by the farmer at 70 kg/ha only at the time of sowing. The fertilizer was Macro Pro Extra fertilizer (CSBP

Table 1. Cropping rotations and legumes species studied in the south-west agricultural area of Western Australia

	Region	Preceding pasture legume	N treatments	The crop or crop sequence, from which data was obtained
Legume *v.* fallow				
Experiment 1	Esperance	Cadiz	Legume N, fallow, 5 rates of inorganic N	Wheat (Mace)
Experiment 2	Mount Vernon	Margurita	Legume N, fallow, 4 rates of inorganic N	Canola (Yetna)
Multiple year response				
Experiment 3 A	Chapman Valley	Biserrula	Legume N plus 5 rates of inorganic N	Wheat (Mace)
Experiment 3 B	Chapman Valley	Biserrula	Legume N plus 5 rates of inorganic N	Canola, Wheat (Mace)
Experiment 3 C	Chapman Valley	Biserrula	Legume N plus 5 rates of inorganic N	Oats, Canola, Wheat (Mace)
Experiment 4 D	Scaddan	Annual medics	Legume N plus 5 rates of inorganic N	Wheat (Mace)
Experiment 4 E	Scaddan	Annual medics	Legume N plus 5 rates of inorganic N	Barley (La Trobe)
Single year response				
Experiment 5.1	Cunderdin	Subterranean	Legume N plus 5 rates of inorganic N	Wheat (Mace)
Experiment 5.2	Brookton	Clover	Legume N plus 5 rates of inorganic N	Wheat (Mace)
Experiment 5.3	Dandaragan	French serradella	Legume N plus 5 rates of inorganic N	Wheat (Calingiri)
Experiment 5.4	Cascade	Serradella mix	Legume N plus 5 rates of inorganic N	Barley (La Trobe)
Experiment 5.5	Corrigin	French serradella	Legume N plus 5 rates of inorganic N	Wheat (Mace)
Experiment 5.6	Babakin	French serradella	Legume N plus 5 rates of inorganic N	Wheat (Mace)
Experiment 5.7	Ardath	Bladder clover	Legume N plus 5 rates of inorganic N	Wheat (Mace)
Experiment 5.8	Varley	Biserrula Biserrula	Legume N plus 5 rates of inorganic N	Wheat (Mace)

Fertilizers) and it contained 9.7% N, (equivalent to 6.8 kg/ha), 11.2% P, 11.2% K, 9.8% S and 0.10% Zn.

Nitrogen strips (5 × 20 m) were applied as urea at the N rate of 0, 23 (50 kg/ha Urea), 46 (100 kg/ha Urea), 69 (150 kg/ha Urea) and 92 kg/ha (200 kg/ha Urea) on 3rd of June 2014 at tillering stage (Zadoks growth 21–26) (Zadoks *et al.*, 1974) across the crop and fallow treatments. Commercial N rates in the area are 50 Kg/ha of N at tillering followed by an application of 50 Kg/ha of N in middle of the winter.

The N treatments were arranged in a randomized block design with three replicates, across the two main-treatments: serradella and chemical fallow. Seed yield components were measured from material collected from two randomly placed open quadrats (0.5 m^2) in each plot/treatment on the 27th of November 2014.

The site was sampled before seeding and soil analysis was conducted by CSBP Ltd (Bibra Lake, Western Australia) https://www.csbplab.com.au/faq/downloads/downloads.

Experiment 2. Mount Vernon

Experiment 2 was established at Mount Vernon, Western Australia (32°48′ 00.36S, 119°13′ 57.36E) in 2017 to investigate a canola crop response to organic N fixed by hardseeded French serradella Margurita grown in 2016. A chemical fallow treatment was included in the experiment and an additional four rates of N were applied across the treatments.

Pasture phase

On the 15th of February 2016 the paddock (280 ha) was ‘summer sown’ to Margurita at the rate of 20 kg/ha of pods. Pods of Margurita were sourced from header harvested material with minimal post-harvest processing resulting in a low level of germinability (<10%). They were drilled into cereal stubble (dry soil) at 1 cm depth, using no-till machinery with knife points followed by press wheels at row a spacing of 25 cm (Nutt *et al.*, 2021*b*). Pods were inoculated with 10 kg/ha of ALOSCA® granules, group S for serradella. At sowing, 100 kg/ha of superphosphate and 35 kg/ha muriate of potash were applied. Total above-ground biomass of serradella was measured in spring (at estimated peak biomass) on the 23rd of September 2016 from grazed and ungrazed (exclusion cages) areas. Samples were cut to ground level with knives from eight randomly placed open quadrats (0.1 m^2) for each treatment.

Samples were oven dried for 48 h at 60°C to allow calculation of dry matter content. The samples were analysed for N, P, K, S, Ca, Mg, Na, Cu, Zn, Mn, Fe, B by using inductively coupled plasma spectroscopy at the CSBP Soil and Plant Analysis Laboratory (https://www.csbplab.com.au/faq/downloads/downloads). The paddock was planted with the intention of harvesting the seed, therefore it was grazed from May to the end of July with 2.8 DSE/ha, this to allow sufficient biomass regrowth and adequate seed production.

To simulate a fallow treatment, within the area of serradella, two strips of 20 × 100 m were not sown to serradella and sprayed twice with glyphosate 1 L/ha (540 g ai/l) on the 15th of June and 20th of October 2016.

Soil sampling

The site was sampled before seeding with wheat on the 20th of April 2017. To further investigate the effect of factors such as soil pH and soil nutrients levels a comprehensive soil analysis was conducted at the site. Five cores were collected from depths 0–10, 10–20, 20–30 and 30–40 cm with a 50 mm diameter soil auger for each treatment/replicate. Soil analysis was conducted by CSBP Ltd (Bibra Lake Western Australia).

Crop phase

The paddock was sown to Yetna canola at 4 kg/ha of seed on the 28th of April 2017, using no-till machinery with knife points followed by press wheels at row spacing of 25 cm. Fifty kg/ha of basal fertilizer (9.7% N, 11.2% P, 11.2% K, 9.8% S and 0.10% Zn) was applied to ensure that N was the only nutrient element limiting canola production. Seed was treated before sowing with Thiamethoxam 210 g/l and Lambda-Cyhalothrin 37.5 g/l to protect from insect damage prior to emergence. Additional control of insect pests was achieved with post-emergence pesticides (Chlorpyrifos 500 g/l and Bifenthrin 250 g/l).

Weeds were controlled by treatment herbicides before seeding (1.5 l/ha of Glyphosate, 1 kg/ha of Simazine and 1 kg/ha of Atrazine) and at post emergence (0.5 L/ha Clethodim and 1 kg/ha of Atrazine). Nitrogen treatments were applied at 0, 23, 46 and 69 kg/ha on the 1st of June 2017 as urea (N 46%) and top-dressed in a single dose at the rosette stage.

The design was a criss-cross design of the two treatments, serradella and fallow randomized in four main plots (20 by 60 m) crossed with the three banks or replicates, where the intersection of these 12 plots was a split-plot (four sub-plots) with the four N rates for a total of 48 sub-plots (5 by 20 m).

Multiple-year response

Two farms with a long tradition of growing biserrula and annual medics in the wheat belt area were identified. Two crop rotation experiments were conducted on these farms to assess the long-term (more than one year) effects of N fixed in legume pastures on cereal crop yields. The experiments were carried out in the Chapman Valley region (Experiment 3, three sites) and Scaddan region (Experiment 4, two sites) where crop yield was assessed for one year of a cropping sequence (either 1-pasture:1-crop; 1:2; or 1:3), during the three years that followed a single pasture phase. This represents three different rotations between pasture and crops and provided the opportunity to study the effect of N fixed in a single year by the legume to the crops in following years. Five N rates were applied, replicated three times, to establish the organic N supplied by the preceding pasture and determine whether there was any N response.

Experiment 3 – Chapman valley region

The experiment was located in the northern part of the Western Australia wheat belt at Chapman Valley, considered a medium rainfall area (440 mm mean annual rainfall), 50 km northeast of Geraldton (28°34′ 28.97″S, 114°45′ 28.96″E). Three adjacent paddocks (site A, B and C, each 500 m apart), within the same farm were chosen to carry out the investigation. Site A was sown for the first time to biserrula (Casbah) in 2015. Total above-ground biomass of biserrula was collected in spring at peak biomass on the 1st of September 2015 from material cut with knives from four randomly placed open quadrats ($0.1\ m^2$) in the area where the 2016 experiment was established. Site B was sown for the first time to biserrula in 2014, then cropped with canola in 2015. Site C was sown for the first time to biserrula in 2013, then cropped with oats in 2014 and cropped with canola in 2015.

The farmer used minimal amounts of N fertilizer on his crops in 2014 and 2015 for site (B) and (C), adding 20 kg/ha of N to the crops (5 kg/ha of N with a starter compound fertilizer, and 15 kg/ha in a form of urea later in the season at early tillering time) compared to the commercial recommended rate of 70–100 Kg of N.

The three sites were sown on the 25th of May 2016 to wheat (Mace), with 25 cm row spacing, after the paddock was sprayed with non-selective herbicide (glyphosate 1 L/ha (540 g ai/l)). No fertilizers were added for the entire growing season by the farmer.

On the 14th of June 2016, N treatments were applied as urea at tillering stage (Zadoks 21–26) at the N rate of 0, 23, 46, 69, 92 kg/ha of N. As for Experiments 3 and 4 treatments were arranged in a randomized block design with three replicates.

Experiment 4 – Scaddan region

The Experiment 4 was located in the southern part of the WA wheat belt at Scaddan, a medium rainfall area (450 mm mean annual rainfall), 70 km north of Esperance. Two adjacent paddocks (20 m apart) within the same farm were chosen. The first paddock (site D) (33°24′18.65″S, 121°31′08.22″ E) was cropped to wheat in 2016, after a year of mixed annual medic pasture (*Medicago truncatula* and *M. polymorpha*), originally sown 40 years earlier and managed since in a rotation of 2 crops to 1 regenerating pasture. The paddock had been managed in a rotation of two crops: then one pasture for the preceding 20 years. The second paddock (site E) (33°24′13.49″S,121°31′08.22″E), with a similar pasture background as site (D), was cropped to wheat (Mace) in 2014 and 2015, and then cropped to barley in 2016 to simulate a 3:1 crop pasture rotation. The farmer used minimum amounts of N artificial fertilizer in 2014 and 2015 for site (E), adding only 20 kg/ha of N (5 kg/ha with a starter fertilizer (as in Experiment 1 in Esperance), and 15 kg/ha later in the season at tillering stage in a form of urea).

The two sites were sown on the 19th of May 2016 to wheat (Mace) and barley (La Trobe) at sowing rate of 70 kg/ha, with 25 cm row spacing, after the paddock was sprayed with non-selective herbicide (glyphosate 1 l/ha (540 g ai/l)).

On the 22 of June 2016, N treatments were applied with urea at tillering (Zadoks growth stage 21–26) at rates of 0, 23, 46, 69 and 92 kg/ha of N. Plots were organized in a randomized block design.

Dry matter yield of the medics was not recorded in 2015, however the researchers had worked in the farm during the previous five years and estimated an average dry matter yield between 5 and 7 t/ha. The site was grazed during the pasture phase.

Soil sampling

All sites were soil sampled once, in May 2015 before sowing the crops. Each block was divided into quarters and five cores were collected from each quarter from depths 0–10, 10–20, 20–30 and 30–40 cm with a 50 mm diameter soil auger. Individual cores were combined to give bulk samples from the site for each depth. Soil analysis was conducted by CSBP Ltd (Bibra Lake, Western Australia). The three sites were acidic pH (H_2O) decreasing in depth and ranging from 6.4 to 5.6 on surface and from 6.3 to 5.2 at 30–40 cm depth (Table 2).

Crop sampling

Crop biomass cuts were taken on the 21st of September for the Chapman valley site and on the 6th of October at Scaddan (Zadoks growth stage 50–60) from two randomly placed open

Table 2. Chapman Valley Soil characteristics at 0–40 cm depth for the 3 experimental sites, sampled in autumn before crop establishment

Site (A)	0–10 cm	10–20 cm	20–30 cm	30–40 cm	Avg RSD ($n = 3$)
pH (H_2O)	6.4	6.0	6.0	6.3	0.24
pH ($CaCl_2$)	5.8	5.4	5.3	5.5	0.21
Ammonium N (mg/kg)	1.3	1.0	1.0	1.0	0.29
Nitrate N (mg/kg)	22.7	26.7	19.3	8.3	2.71
Phosphorus (mg/kg)	33.3	25.7	14.0	9.3	3.61
Potassium (mg/kg)	276.7	239.0	222.0	204.3	18.31
Sulphur (mg/kg)	10.3	19.9	40.2	31.7	6.70
Organic Carbon (%)	0.71	0.48	0.37	0.33	0.06
Site (B)					
pH (H_2O)	5.6	5.3	5.2	5.2	0.14
pH ($CaCl_2$)	4.7	4.7	4.6	4.7	0.12
Ammonium N (mg/kg)	2.3	1.7	1.7	1.0	1.30
Nitrate N (mg/kg)	13.0	14.0	11.7	6.7	3.19
Phosphorus (mg/kg)	43.3	32.7	15.3	7.0	7.47
Potassium (mg/kg)	154.7	104.7	94.3	103.7	11.26
Sulphur (mg/kg)	6.5	6.8	13.1	28.9	4.09
Organic Carbon (%)	0.62	0.31	0.19	0.20	0.08
Site (C)					
pH (H_2O)	6.1	5.8	5.4	5.6	0.36
pH ($CaCl_2$)	5.5	5.1	4.8	4.9	0.34
Ammonium N (mg/kg)	5.7	3.7	1.7	1.3	2.02
Nitrate N (mg/kg)	35.7	27.7	20.3	11.3	3.37
Phosphorus (mg/kg)	32.0	35.7	17.3	9.0	4.84
Potassium (mg/kg)	182.3	157.0	130.0	122.7	11.49
Sulphur (mg/kg)	9.8	11.5	21.1	26.1	5.13
Organic Carbon (%)	0.66	0.47	0.27	0.28	0.08

Experiment 3 (A,B,C) – Multiple-year response.

quadrats (0.1 m^2) in each plot and dried at 60°C. Wheat seed yield components were collected on 1st of November at Chapman Valley and on the 23rd and 24th of November at Scaddan from two randomly placed open quadrats (0.5 m^2) in each plot/treatment. Number of heads, total grain yield and thousand seed weight were measured.

Single year response

The single-year N response experiments 5.1 to 5.8 compared the crop response with the establishment of a range of pasture legumes at different locations in the mixed farming region of Western Australia. Eight experiments, were conducted where a range of annual legume pastures (subterranean, rose and bladder clovers, yellow and French serradella and biserrula) were established by the host farmers and managed under commercial conditions, followed by a crop in the second year. In the crop year, the crops were sown as part of the normal seeding programme, and then 5 N rates were applied as randomized block design replicated three times.

This study was carried out across eight sites to investigate crop response to organic N provided by several pasture legumes grown in the antecedent year. The first five sites (2013) were located in WA at Cunderdin: 31°38′39.17″S, 117°20′18.88″E (sandplain), Brookton: 32°14′56.84″S, 116°49′55.18″E (loamy sand), Dandaragan: 30°49′13.49″S, 115°49′36.49″E (sandy), Cascade: 33°33′30.013″S, 121°13′17.95″E (sandy) and Corrigin: 32° 17′47.60″S, 117°39′48.43″E (sandy).

Two sites were selected in 2015 and were located at Babakin: 32°10′1.74″S, 117°57′15.81″E (loamy sand), Ardath: 32° 4′49.01″S, 118°4′ 57.26″E (loamy sand) and one site in 2016 at Varley: 32°52′54.30″S, 119°25′01.25″E (clay loam). The crops followed one year of bladder clover and two of biserrula, respectively. N treatments were the same as in Experiment 1 (0, 23, 46, 69, 92 kg/ha of N) and were applied on the 10th and 14th of June in 2013, on the 20th of June in 2015 and on the 22nd of June 2016 on plots of 5 × 10 m. Seed yield components were measured from material collected from two randomly placed open quadrats (0.5 m^2) in each plot/treatment from the 13th to the 20th of November 2013, on the 10th of November 2015 and on the 23rd of November 2016. The treatments were allocated in a randomized block design with three replicates. All sites were soil sampled before sowing the crops.

Statistical analysis

The Genstat Statistical software (https://vsni.co.uk/software/genstat) was used to conduct statistical analyses for each of the experiments. Statistical analyses with P-value <0.05 were accepted as significant results.

Legume v. fallow

A combined analysis of variance for a criss-cross type analysis was performed for each of the measurements using REML to compare the N treatments within and between the main serradella and chemical fallow strips.

Multiple and single-year response

Orthogonal polynomials (linear and quadratic trends) were examined for the experiment measurements using the ANOVA procedure in Genstat for each of the experiments.

Statistical analysis of soil measurements for Experiment 2 in the Legume *v*. Fallow and multiple-year response experiments was conducted using a linear mixed model fitted using the REML procedure to estimate the mean depth responses for pH and nutrients and the average residual standard deviation (average RSD).

Results

Legume v. fallow

Experiment 1. Esperance

The wheat yield from the no added N after serradella treatment (legume N) was higher ($P < 0.1$) than the no added N after fallow treatment (3.4 *v* 2.5 t/ha) (Table 3). Within the serradella treatments there was no difference between the yield of the no added N treatment and the yields related to the additional rates of N ($P > 0.1$).

In the fallow treatment, only the highest rate of N was different ($P < 0.1$) from the no added N treatment (Table 3). Protein levels increased ($P < 0.1$) by increasing the N rates across the pasture legume and fallow treatments. They ranged from 11.5 to 14.1% in the serradella treatment and from 12 to 13.4% in the fallow treatment.

During the pasture phase in 2013, the dry matter yield of French serradella (Cadiz) averaged 5.5 ± 0.3 t/ha (mean ± SE) and the N content at peak biomass was 3.2%. Soil analysis conducted in autumn before crop establishment presented a higher content of N in the serradella treatment compared to the fallow (22.3 and 9.0 kg/ha respectively) (Table 4).

Experiment 2. Mount Vernon

The annual rainfall at Mount Vernon for 2017 was of 372 mm, with 188 mm received in the growing season (May to October) (Table 5). The site was moderately acidic ranging from pH 6.0 on the surface to slightly higher at 30–40 cm with values of pH 6.8 in the serradella treatment (legume N) and pH 6.5 in the fallow treatment (Table 6).

Within the serradella treatment, canola grain yield was lower ($P < 0.05$) at higher N fertilizer rates (2.1 t/ha no added N to 1.6 t/ha for the highest N rate (69 kg/ha)) (Table 7). Grain yield of the no added N after serradella treatment (2.1 t/ha) was different ($P < 0.05$) from the no added N after fallow treatment (1.5 t/ha). The oil percentage did not differ ($P > 0.05$) with varying N application levels after serradella and ranged from 42.7 to 44.5%. The lowest yielding fallow treatments (nil N, 23 and 46

Table 3. Mean wheat grain yields and protein percentage in 2014 after French serradella or chemical fallow

		Nitrogen (kg N ha^{-1})					LSD ($P = 0.1$)	LSD ($P = 0.1$)
Main treatment	Response	0	23	46	69	92	Within Main	Between Main
Serradella	Yield (t/ha)	3.4	3.7	2.8	3.2	3.7	0.62	0.77
	Protein %	11.5	12.3	13	13.4	14.1	0.67	1.02
Fallow	Yield (t/ha)	2.5	3.1	2.9	3.0	3.4	0.62	0.77
	Protein %	12.0	12.1	11.9	13.2	13.4	0.67	1.02

Experiment 1.

Table 4. Soil characteristics at 0–10 cm depth

Site	Esperance (Fallow)	Esperance (Serradella)	Cunderdin	Brookton	Dandaragan	Cascade	Corrigin	Babakin	Ardath	Varley
pH (H_2O)	5.8	5.9	5.6	5.9	6.2	7.2	5.9	6.0	6.3	5.9
pH ($CaCl_2$)	4.8	4.8	4.9	5.3	5.5	6.5	4.9	5.0	5.7	5.2
Ammonium N (mg/kg)	2	2.1	8.0	3.0	1.0	2.0	2.0	3.0	1.9	1.3
Nitrate N (mg/kg)	7	20.3	34	76	21	35	6.0	4.0	11.3	3.0
Phosphorus (mg/kg)	15	13	30	72	27	10	15	34	22.0	47.0
Potassium (mg/kg)	62	65	77	80	45	82	42	102	117	135.0
Sulphur (mg/kg)	3.8	4.1	13	41	7.7	6.1	6.3	6.8	6.0	55.8
Organic Carbon (%)	1.32	1.35	1.17	1.86	1.14	1.07	1.02	1.15	0.99	0.78

Experiment 1-Legume *v*. Fallow, experiments 5.1 to 5.8 – Single year response.

Table 5. Total monthly rainfall (mm) during the experimental period (data from Australian Government, Bureau of Meteorology) for all experiments sites

Site	Year	Experiment	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Esperance	2013	1	11.4	12.2	5.2	11.0	65.4	50.0	93.2	51.0	46.6	79.6	71.6	23.0	520
Mt Vernon	2017	1	26.6	75.2	23.6	4.4	14	25.2	54.2	51.6	43.2	30.8	15	8.4	372
Cunderdin	2013	2	53.8	3.0	41.6	15.6	46.4	12.6	63.6	65.8	76.6	34.0	1.2	53.8	449
Brookton	2013	2	22.6	0.6	55.6	5.0	67.6	21.4	111	73.6	103.4	24.8	1.0	3.8	490
Dandaragan	2013	2	31.0	0.0	41.4	18.0	74.6	9.0	60.2	72.8	64.0	17.6	0.6	1.0	390
Cascade	2013	2	17.6	4.0	221	39.4	63.0	25.4	51.2	31.8	46.4	53.4	11.8	35.4	600
Corrigin	2013	2	89.3	0.2	28.5	3.5	53.1	3.3	112	53.2	57.0	23.2	2.9	17.6	444
Babakin	2015	2	0.0	1.2	34.4	12.6	16.6	32.0	32.8	59.6	12.4	3.2	26.7	7.2	239
Ardath	2015	2	0.4	5.6	43.6	18.8	11.8	38.4	46.8	80.2	9.2	1.3	38.0	5.2	299
Varley	2016	2	79.8	7.2	86.8	15.2	32.6	45.4	39	61.2	23.6	10.6	4.6	24.4	430
Chapman Valley	2013	3	12.0	0.0	39.6	2.2	84.8	3.8	40.4	69.4	54.0	23.4	0.0	0.0	329
Chapman Valley	2014	3	9.8	1.2	0.6	56.0	67.6	31.0	63.0	17.0	72.8	3.6	5.0	0.0	327
Chapman Valley	2015	3	3.0	3.6	89.0	42.8	0.0	50.6	85.3	46.0	13.6	2.8	25.3	3.8	365
Chapman Valley	2016	3	26.8	0.0	11.0	46.9	31.0	72.8	112	67.8	20.8	11.2	5.4	0.4	406
Scaddan	2013	3	38.2	11.0	139	21.5	56.6	28.0	0.0	37.4	36.6	0.0	0.0	17.0	385
Scaddan	2014	3	0.0	11.0	0.0	13.2	35.0	21.8	37.4	26.5	33.1	59.0	42.7	18.5	298
Scaddan	2015	3	0.0	0.0	44.0	42.4	31.4	50.0	37.9	64.7	23.2	25.2	22.8	32.3	373
Scaddan	2016	3	86.5	22.6	70.8	25.0	46.8	72.1	46.2	67.7	49.9	19.6	9.6	5.2	522

Table 6. Soil characteristics at 0–40 cm depth after French serradella and fallow at the Mount Vernon Experiment 2

Rotation	French serradella				Fallow				
Soil depth	0–10 cm	10–20	20–30	30–40	0–10 cm	10–20	20–30	30–40	LSD $P = 0.05$
pH (H_2O)	6.0	6.1	6.5	6.8	6.1	6.1	6.4	6.5	0.3
pH ($CaCl_2$)	5.1	5.1	5.6	6.0	5.1	5.2	5.5	5.9	0.2
Ammonium N (mg/kg)	3	2	1	1	2	2	1	1	0.8
Nitrate N (mg/kg)	35	18	8	7	24	13	6	5	5.6
Phosphorus (mg/kg)	28	17	8	4	24	17	8	3	8.1
Potassium (mg/kg)	66	42	36	41	44	42	37	40	8.9
Sulphur (mg/kg)	7	5	3	3	7	5	3	3	1.8
Organic Carbon (%)	1.03	0.52	0.32	0.22	0.97	0.51	0.25	0.18	0.12

Table 7. Mean canola grain yields and oil percentage following one year of serradella or chemical fallow at Mount Vernon

		Nitrogen (kg N ha^{-1})				LSD $P = 0.05$	LSD $P = 0.05$
Main treatment	Response	No added	23	46	69	Within Serradella or fallow	Between Serradella & fallow
Serradella	Yield (t/ha)	2.10	1.65	1.77	1.60	0.33	0.29
	Oil %	44.5	44.1	43.4	42.7	2.20	2.44
Fallow	Yield (t/ha)	1.48	1.68	1.90	1.77	0.33	0.29
	Oil %	47.2	48.3	47.2	44.7	2.20	2.44

Experiment 2 – Legume *v.* Fallow.

N rates) contained the highest oil content (47.2, 48.3, 47.2% respectively).

Dry matter yield at peak biomass in spring of French serradella (Margurita) in the pasture phase (2016) averaged 5.1 ± 0.14 and 10.0 ± 0.30 t/ha in the grazed and ungrazed treatments, respectively. The total N content of plant material at sampled at peak biomass was 3.6% (SE 0.08) for both treatments. The unsown strip was treated as a chemical fallow, and no dry matter of any species was produced during the year.

The total quantity of plant-available soil N recorded for the serradella and for the fallow treatments in autumn before crop establishment was respectively 75 and 54 mg/kg of soil. The other three components P, K and S were measured at adequate levels for crop production. Organic C in the first 20 cm was 1% (Table 6).

Multiple year response

Experiment 3 – Chapman Valley region

Dry matter yield of the wheat Mace crops at each N application rate for the three sites (A, B and C) is reported in Table 8. In 2016 wheat grain yields averaged 5.0 t/ha with 10.7% protein and there were no differences ($P > 0.05$) among the yields associated with N application rates ($P > 0.05$). (Table 8).

Dry matter yield of biserrula (Casbah) in spring 2015 averaged 6.3 ± 0.2 t/ha. The grazing of the site was organized by the farmer and the paddock was grazed with sheep at 8 DSE ha^{-1} from July to September.

Site (B) was sown to biserrula in 2014, followed by canola in 2015 and sown to wheat in 2016. The dry matter yield of biserrula was not recorded, however the farm has been monitored for the last 15 years and biserrula dry matter yields have constantly ranged between 5 and 7 t/ha. Grain yields after one year of biserrula (legume N) averaged 5.0 t/ha in 2016 and there were no significant differences in grain yield associated with the N application rates. Number of heads m^2, protein % and 1000 seed weight did not differ among the N rates ($P > 0.05$; Table 8).

Site (C) was sown to biserrula in 2013, followed by oats in 2014, canola in 2015 and wheat in 2016. In 2016 there were no differences ($P > 0.05$) associated with N application rates on wheat yield and protein level s. The average grain yield was 4.4 t/ha, 10.7% for the protein, 313 heads/m^2 and 44.7 g per 1000 seeds. (Table 8). The biserrula dry matter yield was not recorded for this site.

Wheat dry matter yields (Zadoks 55) at the 3 sites were not different ($P > 0.05$) between no added N controls and the higher rates of N ($P > 0.05$). The dry matter yield for the no added N treatments at the three sites was of 6.3, 5.0 and 4.7 t/ha respectively (Table 8).

The annual rainfall ranged from 329 mm in 2013 to 406 mm in 2016 (Table 5). Total amount of N recorded for the three sites (A, B,C) in autumn before crop establishment was of 81.3, 45.4 and 113.7 mg/kg of soil in the upper soil profile (0–40 cm), respectively. The other three components P, K and S were detected at adequate level for crop production with site (A) showing high potassium levels over 200 mg/kg in the first 10 cm. Organic carbon levels were similar for the three sites and ranged from 0.7% in the first 10 cm to 0.2% at 30–40 cm (Table 2).

Table 8. Grain yield components of wheat (Mace) and dry matter yields at Zadock 55 stage at the 3 sites in Chapman Valley in rotation with biserrula

Site		Chapman Valley Site (A)					Chapman Valley Site (B)					Chapman Valley Site (C)			
Rotation		Biserrula 2015 Wheat 2016					Biserrula 2014 Canola 2015 Wheat 2016					Biserrula 2013 Oats 2014 Canola 2015 Wheat 2016			
N rates kg/ha	Dry matter *	Heads	Yield	Prot.	1000 sw	Dry matter	Heads	Yield	Prot.	1000 sw	Dry matter	Heads	Yield	Prot.	1000 sw
	t/ha	m^2	t/ha	%	Gr	t/ha	m^2	t/ha	%	Gr	t/ha	m^2	t/ha	%	gr
0	6.3	320	5.1	10.1	43.0	5.0	297	5.4	11.3	44.5	4.7	313	4.4	10.7	44.3
23	6.3	283	5.2	10.8	45.1	4.7	303	5.2	11.4	41.7	4.5	292	4.4	10.7	44.7
46	5.6	294	4.7	11.2	42.3	4.9	290	5.0	11.3	45.1	5.2	306	4.2	10.7	45.3
69	6.3	257	5.1	10.1	41.1	5.0	308	4.9	11.6	40.2	4.9	334	4.4	10.9	44.5
92	5.8	304	5.1	11.2	41.2	5.0	272	4.7	11.8	42.2	5.0	320	4.8	10.7	44.6
L.S.D. ($P = 0.05$)	0.9	49	0.8	0.4	2.4	0.7	47	0.9	0.5	4.1	1.2	52	1.2	0.9	2.9

Experiment 3 (A,B,C) – Multiple-year response.
*Dry matter cuts at Zadock 55 stage.

Experiment 4 – Scaddan region

In 2016 wheat grain yields averaged 4.3 t/ha with 11.8% protein and there were no differences ($P > 0.05$) in yield associated with the N application rates. Protein levels differed ($P < 0.05$) between the no added N control and the highest N rate (10.9% *v* 12.9%) (Table 9).

The barley grain yield (site (E) averaged 4.8 t/ha, with difference ($P > 0.05$) between the no added N treatment (legume N) and the highest N application rates. Protein levels and the other yield components did not differ ($P > 0.05$) among treatments (Table 9).

At the two sites wheat and barley dry matter yields (Zadoks 55) were not different ($P > 0.05$) between no added N controls and increased rates of N. The average dry matter yield for the two sites was of 7.4 and 7.2 t/ha respectively (Table 9).

The region in 2016 experienced a wet summer with a total annual rainfall of 522 mm when compared to the long-term annual mean of 385 mm. Over 200 mm were measured between January and April (Table 5).

Soil type at the two sites (D and E) was typical Scaddan soil, with sand over clay and pH ranging from 8.4 on the surface to 9.7 at 30–40 cm depth. The two sites were uniform as reflected by the RSD for the different components of the soil analysis conducted in autumn before crop establishment (Table 10). Measured N for the two sites was 126.1, 109.4 mg/kg of soil respectively. The level of Phosphorous at the site (D) and site (E) ranged between 19.0 and 39.7 mg/kg respectively. Potassium levels in the first 10 cm of soil were above 600 mg/kg at both sites. Organic carbon levels were similar for the two sites and ranged from 1.7 to 2.0% in the first 10 cm, and from 0.7 to 0.9% at 30–40 cm (Table 10).

Single year response

Experiment 5.1 to 5.8

Grain yields of the eight sites ranged between 1.9 and 5.4 t/ha (Table 7). The lowest yields were recorded at Corrigin and Babakin which also experienced below-average rainfall for the year (Table 5). At all sites, wheat yield and protein level did not differ ($P > 0.05$) due to N application treatments, with the exception of the protein level at the Babakin and Ardath sites which increased from 11.0 to 16.2% and 13.9 to 15.9% respectively ($P < 0.05$) among N rates (Table 11). In 2015 at the Varley site, wheat grain yield averaged 3.2 t/ha and protein levels 10.9%. There was no difference ($P > 0.05$) among N rates (Table 11). Biserrula peak biomass in 2015 was 4.0 t/ha (SE 0.3) with the site lightly grazed during the winter. Soil analysis was conducted for all sites in autumn before crop establishment (Table 4).

Discussion

This study evaluated our hypothesis that pasture legume phases (with serradella, biserrula and bladder clover) can provide sufficient N for subsequent crops to maintain yield and quality without inorganic N application. Our data provided evidence that grain yields of crops grown after the newly commercialized legumes compared favourably with the yields obtained following application of fertilizer N. Across all sites, we consistently found no increase in wheat yield in response to the applications of extra fertilizer N when grown after a pasture legume. This indicates that antecedent legumes provided enough N to grow a crop to its maximum potential for the given year (Tables 3, 7, 8, 9 and 11).

Table 9. Grain yield components and dry matter yields at Zadock 55 stage of wheat and barley at 2 sites at Scaddan

Site	Sc. Site (D)					Sc. Site (E)				
Rotation	Medics 2015 Wheat 2016					Medics 2013 Wheat 2014 Wheat 2015 Barley 2016				
N rates kg/ha	Dry matter*	Heads	Yield	Prot.	1000 sw	Dry matter	Heads	Yield	Prot.	1000 sw
	t/ha	m^2	t/ha	%	gr	t/ha	m^2	t/ha	%	Gr
0	7.0	280	4.6	10.9	45.9	6.9	469	5.4	11.1	39.5
23	7.4	248	4.3	11.2	44.6	7.2	441	4.9	10.9	39.9
46	6.7	268	4.1	11.7	45.1	7.6	502	5.2	10.6	39.0
69	7.9	254	4.2	12.2	43.1	7.3	445	4.7	12.2	37.3
92	7.8	339	4.3	12.9	46.0	7.1	432	4.0	12.5	35.8
LSD ($P = 0.05$)	1.4	76	0.7	1.5	2.9	0.9	89	0.8	2.0	3.4

Experiment 4 (D, E) – Multiple-year response.
*Dry matter cuts at Zadock 55 stage.

Table 10. Scaddan soil characteristics at 0–40 cm depth, sampled in autumn before crop establishment

Site (D)	0–10 cm	10–20	20–30	30–40	Avg RSD ($n = 3$)
pH (H_2O)	8.7	9.3	9.7	9.7	0.20
pH ($CaCl_2$)	7.8	8.3	8.5	8.5	0.15
Ammonium N (mg/kg)	1.7	1.7	1.7	1.3	0.67
Nitrate N (mg/kg)	22.3	29.3	30.7	25.7	3.48
Phosphorus (mg/kg)	19.0	11.0	5.3	8.0	1.68
Potassium (mg/kg)	608.0	442.3	432.3	507.0	127.
Sulphur (mg/kg)	4.7	7.3	12.3	22.7	2.19
Organic Carbon (%)	1.74	0.98	0.63	0.73	0.18
Site (e)					
pH (H_2O)	8.4	8.9	9.4	9.6	0.35
pH ($CaCl_2$)	7.6	7.9	8.3	8.3	0.21
Ammonium N (mg/kg)	1.7	1.7	1.3	1.0	0.50
Nitrate N (mg/kg)	28.7	20.3	24.0	30.7	6.40
Phosphorus (mg/kg)	39.7	20.3	14.0	11.3	6.84
Potassium (mg/kg)	748.7	489.3	551.0	657.0	97.27
Sulphur (mg/kg)	5.6	4.3	6.0	14.0	4.09
Organic Carbon (%)	1.98	1.40	1.09	0.91	0.27

Experiment 4 (D, E) – Multiple-year response.

Peoples *et al.* (1998) indicated that 20–25 kg of N is fixed for every 1 t of dry matter produced by a pasture legume and that 30% of this total N is available the year after the legume phase. Based on this calculation, the total fixed N available from the productive pastures in our study would approximate 50 kg/ha. In our study, the pasture legume species produced over 5 t/ha of dry matter (Legume *v.* Fallow study), which provided approximately 50 kg/ha of N to the following crops. A wheat crop yielding three tonnes of grain, is predicted to require around 70 kg of N, depending on soil N levels (Harries *et al.*, 2020). Based on our results it is feasible that a productive pasture that is dominant in annual pasture legumes and grown in the low to medium rainfall farming region would be able to meet the N requirements of a subsequent cereal or canola crop under average growing conditions. This is an important outcome for those wishing to reduce the input costs and carbon footprint of cropping, with substantive

Table 11. Grain yield and protein of wheat at 7 sites and barley at 1 site following different pasture legumes

Site	Cunderdin (2013)		Brookton (2013)		Dandaragan (2013)		Cascade (2013)		Corrigin (2013)		Babakin (2015)		Ardath (2015)		Varley (2016)	
Crop	Wheat (Mace)		Wheat (Mace)		Wheat (Calingiri)		Barley (La Trobe)		Wheat (Mace)		Wheat (Mace)		Wheat (Mace)		Wheat (Mace)	
Antecedent legume	Subterranean clover		French serradella		Serradella mix Rose clover		French serradella		French serradella		Bladder clover		Biserrula		Biserrula	
N rates kg/ha	Yield	Prot.	Yield	Prot.	Yield	Prot.	Yield	Prot.	Yield	Prot.	Yield	Prot.	Yield	Prot.	Yield	Prot.
	t/ha	%	t/ha	%	t/ha	%	t/ha	%	t/ha	%	t/ha	%	t/ha	%	t/ha	%
0	4.3	11.8	5.0	9.6	4.6	10.1	4.3	11.2	2.7	10.2	2.3	11.0	3.4	13.9	3.4	10.9
23	4.4	11.0	4.2	10.2	3.9	10.2	5.0	12.1	2.6	9.8	2.4	11.2	3.4	12.5	3.2	10.4
46	4.4	11.6	4.7	10.3	4.2	10.5	4.4	12.8	3.2	10.3	2.0	13.5	2.9	13.8	3.0	11.0
69	4.3	11.8	5.4	11.0	4.5	10.9	4.7	13.9	3.4	11.4	2.2	14.2	3.2	14.1	3.4	11.0
92	4.3	11.5	4.8	10.3	4.6	10.3	4.2	13.3	2.8	10.6	1.9	16.2	2.8	15.9	3.0	11.4
LSD ($P = 0.05$)	0.8	2.1	1.3	1.9	0.9	1.2	1.2	2.5	0.9	2.0	0.5	0.7	0.6	0.7	0.9	1.0

Experiment 5.1 to 5.8 – Single-year response.

CO_2 emissions released from the production, distribution and application of synthetic N fertilizer (Al-Kaisi *et al.*, 2008).

Management of ley pasture legumes has a significant impact on the N benefits provided to the subsequent crop (Bell *et al.*, 2017). A high proportion of urinary N is transferred to the soil where pastures are intensively grazed (Ledgard, 1991). While the supply of N combined with rotation effects reduces input costs for cropping, the main profit driver is a high feeding value for livestock, which extends later in the season in pasture legumes compared with other annual pasture species (Thomas *et al.*, 2021). Therefore, some key criteria are important for profitable pasture legume phases including successful establishment and sward management, maintaining a regenerating seed bank, ensuring high pasture utilization through stocking density and benefits to cropping through improved management of disease and weeds (Thomas *et al.*, 2022). Cropping rotations with pasture phases were found to be around $100/ha more profitable compared with continuous cropping, provided the previously mentioned criteria are met (Thomas *et al.*, 2022). In our experiments, sites were typically grazed during the pasture phase, and N provided to the following crops was net of the removal of a significant amount of biomass by the animals (e.g. 5.0 t/ha DM in Legume *v.* Fallow study, Experiment 2). Studies on the fate of excreta N have shown that most excreta N is deposited in urine, predominantly in the form of urea, which is readily available for plant uptake but is also prone to significant N loss due to the concentrated, localized return. In contrast, the availability of legume N via senescence/mineralization is a relatively slow process and occurs over many years (Ladd *et al.*, 1985).

The previous calculation by Peoples *et al.* (1998) did not consider effects of grazing, however we would expect a substantial amount of N recycling, and potentially greater N fixation under grazed pastures (Peoples and Baldock, 2001). Unkovich *et al.* (1998) investigated the effect of grazing on the capacity of N fixation in swards of subterranean clover, without finding differences in total N accumulation between the lightly grazed (300 kg/ha) and the intensively grazed pasture (302 kg/ha).

The multiple-year study (Experiment 3, sites A, B, C – Chapman valley region) has provided the opportunity to investigate the soil composition and the amount of N available in the soil after a biserrula pasture. One of the ongoing difficulties in understanding the role of legumes in the provision of soil N for subsequent crops is the accuracy of methods to determine the amount of organic N in the soil and the amount of plant-available mineral N likely to be released during crop growth. The breakdown of organic N will depend on out-of-season and in-season rain, temperature and microbial activity after the annual legume phase. Our results demonstrate that soil analysis of plant available N (NH_4 and NO_3) should not be considered as the sole predictor of N availability following a pasture legume in a crop rotation. There are several tools available to assist growers to make informed decisions about their N regime that also take into consideration the legume biomass produced the previous year. Our results indicated that the amount of legume biomass produced the year before is probably the most important factor when pasture legumes are included in the rotation. However, total legume biomass production is often not estimated as this is considered difficult to measure by farmers because of below-ground biomass, the biomass removal due to grazing and the difficulty of maintaining ungrazed areas for comparison. There may be some scope to estimate grazed biomass using calculations based on stocking rates and growth rates or modelling methods, however direct pasture intake measurements in extensive grazing systems at commercial scale is not yet feasible (Galyean and Gunter, 2016). As a consequence, the legume biomass may be underestimated or not recorded properly, and therefore there is the tendency to rely mainly on the data provided by the soil analysis.

The sites at Chapman valley (Experiment 3, sites A, B and C) are an excellent example to describe this discrepancy. Whilst the sites were very similar for most of the soil characteristics, they differed remarkably for the total N levels measured. The paddock that was cropped the most (site C) recorded the highest N level (113.7 mg/kg), compared to site (B) (cropped once) that recorded 52.1 mg/kg and the site (A) (never cropped) that recorded 81.3 mg/kg. However, all three sites recorded grain yields completely unresponsive to any additional N fertilizer rates applied during the season, suggesting that the legume grown previously provided sufficient N up to the third consecutive crop. It should be noted that application of N as urea is prone to losses through volatilization, which may be as high as 50% (Peoples *et al.*, 1995). As the timing and conditions for the application of urea is important for the delivery of N to crops and their subsequent yields, the conditions at the time of application were managed carefully as outlined in the methods, and a range of treatment levels was used to identify any dose response.

During 2016, the northern part of the WA wheatbelt experienced and unusual wet and long spring (Table 5) that generated very high wheat yields for the region, unfortunately associated with general low protein levels. The results of the wheat National Variety Experiments conducted at Nabawa a few km away from our three sites at Chapman Valley, found the protein level of the cultivar Mace (used in our experiments) was 9.2% (NVT, 2016), which was lower compared to the level of our three no added N controls that ranged from 10.1 to 11.3%. Similar results were also found at the two wheat NVT Experiments nearby to the Scaddan site (Experiment 4, sites D and E). Wheat at Gibson and Munglinup produced a 1.5 and 3.2 t/ha yield with protein level of 11.5 and 9.5% respectively; whilst at D – Scaddan, the wheat that followed a rotation with annual medics produced 4.6 t/ha of grain at 10.9% protein without the application of extra N. Therefore, in these low input systems no extra inorganic N applied influenced grain yields nor did it decrease the grain quality significantly.

Conclusions

Due to the high value of N inputs from pasture legumes in rotation, and their productivity in supporting livestock production, pasture legume phases are likely to play more of a role in sustainable low input mixed farming systems than grain legumes and chemical fallows. This study provides strong evidence that one year of a pasture legume in rotation provides sufficient organic N to grow competitive grain and oilseed yields in subsequent seasons. In some cases, we found that pasture legume phases are able to supply N to crops even after the second and third year in the rotation.

Further research is required to identify the most appropriate rotation on specific soil types, and to define the length of the rotation to maximize farm profits without compromising the survival of the pasture legume seed bank (Thomas *et al.*, 2022). Additionally, it will be important to classify the risk mitigation of annual aerial-seeded pasture legumes. Such as providing disease breaks, supporting livestock production, weed and soil moisture conservation management, and decreasing frost effects while absorbing increasing N fertilizer costs in a dryland farming system as the climate becomes more variable.

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