

UNIVERSITY *of*
TASMANIA

Prospects for improving productivity and
composition in pasture/crop rotations by
changing row configuration at sowing

by

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University of Tasmania, November 2021

Declaration of Originality

I declare that this is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been duly acknowledged in the text and a list of references is given.

20 August 2021

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Due to the inclusion of published works there is unavoidable repetition of material between chapters in this thesis.

20 August 2021

Thesis structure

This is a thesis through publication. Chapters 3 and 6 have been published in *Crop and Pasture Science*, Chapter 4 in *Plant and Soil* and Chapter 5 in *Renewable Agriculture and Food Systems*. Chapter 7 has been submitted to the *Journal of Agronomy and Crop Science* and is presently undergoing peer review. A title page has been added to each experimental chapter that includes the citation details. The Introduction, Literature Review and General Discussion have not undergone peer review. For the chapters that have been published, I have retained the formatting of the particular journal, although numbers at the bottom (centre) of each page are contiguous throughout the thesis and are the numbers to which the Table of Contents relate. Each chapter has its own discreet reference list, except for the introductory chapter which shares its reference list with the Literature Review. There is one appendix to this thesis, cited in Chapter 8, which was a paper I presented at the Australian Agronomy Conference in 2017. Some of the findings presented in that paper are only preliminary and described in more detail in the experimental chapters that were published subsequently. However, two small components of the work, an adoptability analysis and a brief economic analysis, are reported only in that conference paper.

Statement of Co-authorship

The following people and institutions contributed to the experimental chapters in this thesis:

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This thesis comprises a large body of field work that was run in conjunction with the national EverCrop Project, 2008-2015. I assumed leadership of the New South Wales (NSW) component of EverCrop following the retirement of my mentor, Dr Brian Dear in 2010 and with a large network of collaborators across southern Australia, we delivered an ambitious program of work, which helped increase our understanding of the opportunities and challenges perennial plants offer cropping systems. The field experiments described herein build upon the experiments established earlier in the EverCrop Program, many of which are described in the special issue of *Crop and Pasture Science* in 2014, (volume 65 issue 10). That earlier research made us question the practical steps that might be taken to improve the establishment and persistence of mixtures, a question at the very heart of this thesis.

To my regret, the scientific outputs presented here have come long after the funding for this work ceased. Nevertheless, I gratefully acknowledge the financial support of the Future Farm Industries Cooperative Research Centre and the Grains Research and Development Corporation (GRDC), which underpinned this uncharacteristically long program of research. I hope that one day in the not-too-distant future the GRDC will once again engage in the research of perennial plants in cropping environments. My employer, the NSW Department of Primary Industries (DPI) is also acknowledged for its ongoing support of my work and, more generally, of its sustained investment into perennial crop and pasture systems. It has been a great place to work for the last 20 years, and the depth and talent of staff across a range of disciplines has enabled it to continue to deliver world-class, inter-disciplinary farming systems research to stakeholders across NSW and beyond.

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Abstract

The challenge of maintaining a diverse and robust mixture of pasture species in the rain-fed, semi-arid environments common across south-eastern Australia has long been a topic of interest to farmers and researchers alike. The competition for scarce water and nutrient resources, particularly during summer, is often a key factor driving the decline of desirable species over a relatively short timeframe. Managing interspecific competition by changing the spatial arrangement of different pasture species at sowing could be a practical solution that requires little increase in expense or management complexity for the farmer. However, few studies have investigated the effectiveness of this strategy in improving the productivity of pasture swards, nor examined the broader implications of this approach to farming systems where pastures are grown in phased rotations with crops. This thesis draws on data from a network of field sites comprising two series of experiments. The first, located in the Riverina region of southern New South Wales (NSW), Australia, compared ten pasture swards containing lucerne (alfalfa; *Medicago sativa* L.), phalaris (*Phalaris aquatica* L.) and/or subterranean clover (*Trifolium subterraneum* L.) in various drill row configurations. The second, located in the Central West region of NSW compared legume establishment under cover crops of wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), canola (*Brassica napus* L.) and lupin (*Lupinus angustifolius* L.) sown in mixed and alternate drill row configurations with lucerne and subterranean clover. These investigations aimed to evaluate the impact of changing row configuration at sowing on pasture productivity and persistence, and examine the legacy of this practice on soil fertility, the microbiome as well as subsequent crop production.

Sowing species into fewer drill rows often led to reduced overall biomass, although results varied from site to site with differences less evident under drier seasonal conditions. Less total biomass was attributable to a reduction in the productivity of the dominant species in the mixture. Herein lies the ultimate challenge when managing competition in a mixture by increasing the spatial distance between species at sowing. On the one hand the spatial separation of species reduced early competition to improve the opportunity for less vigorous species to establish. On the other hand, constraining the dominant species to fewer drill rows limited the productivity of that species, which frequently resulted in reduced overall productivity of the sward. This was because constraining species to fewer drill rows effectively increased the spacing between those drill rows, and the yield of companion species in the intervening rows often could not compensate for the yield reduction of the dominant species. Nowhere was this seen more clearly than in a covercrop scenario where the yield reduction

associated with confining the vigorous winter crop to alternate drill rows was not matched by the production of slow-establishing pasture legume seedlings in every other drill row.

The legacy of row configuration on perennial-based pasture productivity is enduring. All three pasture species tested were observed to remain largely confined to the original drill for the duration of the 3-year pasture phase, although a greater proportion of the self-regenerating subterranean clover was found on the inter-row area (up to 30%) compared to the perennial species (<5%). This was less evident at the hotter and drier sites where <10% of subterranean clover plants were found in the inter-row area. As a result of remaining confined to the drill row, ongoing reductions in the productivity of dominant species were observed for the life of the pasture phase. For example, at sites where lucerne was the dominant species, row configuration that confined lucerne only to alternate rows had lower ongoing productivity compared to where lucerne was sown in every drill row on account of the increased spacing between lucerne rows in the alternate drill row treatment. However, there was evidence that companion species such as subterranean clover benefitted from spatial separation at sowing. Subterranean clover herbage yield and seed production was consistently greater in treatments where it was sown alone in fewer drill rows compared to where it was sown in every drill row with perennial and covercrop species.

In examining the legacy of pasture row configuration, it was clear that regardless of which species were sown, the soil fertility was substantially different immediately under the pasture drill row compared to in the inter-row area. Soil organic carbon (SOC), mineral nitrogen (N), pH and available (Colwell) potassium were all higher on the drill row, plant-available phosphorus was lower and there was a variable response in sulphur with depth. Given the absence of fertiliser application in the drill row at sowing, the spatial differences in fertility at the end of the pasture phase were plant-driven responses, associated with the ongoing concentration of plants on or near the drill row over the life of the pasture. A corresponding difference in soil microbial populations was also observed with a greater abundance and diversity of fungal and bacterial populations on the drill row. This was attributed to the increase in total C concentrations in the surface soil, implicating plant cover as an important driver of improved soil chemical and biological attributes. Ongoing differences in soil chemistry were observed over time following the termination of pastures with herbicide and although some differences between the drill and inter-row areas diminished, levels of SOC remained significantly higher on the pasture drill row (2.34%) compared to between drill rows (2.12%) in the surface 50 mm of soil, 15 months after the pasture was removed, despite a wheat crop being grown in the meantime.

Differences in soil fertility at the end of the pasture phase led to an 11% increase in grain yield in wheat growing on the previous pasture drill row compared to plants growing on the inter-row area. However, this finding is likely to be of little practical significance, because when averaged over the whole plot area, there was no significant difference in yield due to row configuration at any of the three sites tested. Grain size was smaller and protein concentration was higher in wheat following pastures that had a higher lucerne and/or subterranean clover content due to increased N inputs associated with biological N₂ fixation.

Taken together, these results suggest that changing drill row configuration does offer potential to increase the subterranean clover content in mixed pasture swards, which has benefits to grain quality in subsequent wheat crops. However, the broadscale use of changing row configuration at sowing is not recommended due to the high probability of reduced overall productivity. Rather, a targeted approach is required where knowledge of the dominance hierarchy of species in the mixture exists. Perverse outcomes on total productivity will likely be minimised where row spacing is reduced as narrower drill rows would result in a lower yield gap in crops and pastures due to a larger number of drill rows being sown to the dominant species for a given area of land. Narrower drill rows will achieve greater plant coverage over the land area in short-term pasture phases, which has been demonstrated to confer benefits to soil chemical and biological properties.

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Chapter 1

Introduction

Pasture swards containing a diverse mixture of species are a common objective for graziers around the world to sustain grazing livestock. Mixed pasture swards, particularly those that contain species from multiple functional groups, can increase total biomass through enhanced utilisation of resources (Jolliffe 1997, Picasso *et al.* 2011) and through species complementarity; particularly in nitrogen (N)-limiting environments where N-fixing legumes are grown adjacent to non-legumes (Davies 2001, Finn *et al.* 2013). Moreover, livestock production has been shown to benefit from access to multiple feed sources by reducing health disorders associated with an unbalanced diet and through enhanced diet selection (Woodward *et al.* 2008, Newell *et al.* 2020). For these reasons it remains best practice to sow a pasture containing a range of different species (Nurjaya and Tow 2001).

However, diverse pasture swards have been shown to be difficult to maintain in practice. A number of surveys conducted throughout regions of southern Australia have independently demonstrated that despite a mixture of species being sown initially, pasture swards quickly revert to a state that is dominated by one or two sown species, or by undesirable weeds (Kemp and Dowling 1991, Fortune *et al.* 1995, Bowcher 2002, Bowman *et al.* 2004, King *et al.* 2006, Virgona and Hildebrand 2007). Of particular concern is the apparent inability to reliably maintain a robust legume content (Wolfe and Dear 2001) given that N remains a key limitation to pasture production (Donald 1965, Cocks 1980). This is a concern for two reasons. Firstly, reduced soil N availability has direct impacts on protein content of feed, which results in an immediate and direct negative impact on livestock production (Wolfe *et al.* 1980, Carroll *et al.* 1988, Mariotti *et al.* 2008). Secondly, given that N is an essential nutrient for plant production and that productive pasture species typically have high N requirements, a run-down in available soil N would be expected to have negative impacts on the productivity and persistence of the non-legume sward components (Virgona *et al.* 2012), constraining livestock production and reducing N-inputs to subsequent crops where pastures are grown in phased rotations (Peoples *et al.* 2001).

A delicate balance exists between self-regenerating annual and perennial species in permanent or phased pasture swards in rainfed semi-arid environments, where rainfall is episodic (Ward 2006). The challenge in a perennial-based pasture sward is to have the resilience to withstand a decile 1 rainfall year but to be sufficiently productive to utilise

available water resources and maximise biomass growth during a decade 9 year. Relatively few forage species are sufficiently adapted to persist under such seasonally dry yet variable seasonal conditions (Hayes *et al.* 2012), meaning that pasture mixtures in those environments often consist of only a very small number of species. The perennial grass, phalaris (*Phalaris aquatica* L.), perennial legume, lucerne (alfalfa, *Medicago sativa* L.) and self-regenerating annual legume, subterranean clover (*Trifolium subterraneum* L.) are amongst the most persistent and productive pasture species available for semi-arid environments of south-eastern Australia, where rainfall is equi-seasonal or winter-dominant (Kemp *et al.* 2002, Hayes *et al.* 2010a). These are therefore the species that present the best chance of achieving a stable and productive pasture mixture in many environments to which they are adapted (Virgona and Hildebrand 2007, Hayes *et al.* 2012). However, even with these well-adapted species, resilient swards containing all three species over the long-term are relatively difficult to achieve (Dear *et al.* 2001, Hayes *et al.* 2018).

Separating species in different drill row configurations at sowing has been proposed as one strategy that may assist in the successful establishment of multiple species in a mixed sward. This approach was shown by Santhirasegaram and Black (1967) to increase the productivity of pastures in the establishment year planted under a winter cereal covercrop in the last year of a cropping phase (a common practice of many Australian farmers to defray the costs of pasture establishment; McCormick *et al.* (2014)) compared to where the cereal and pastures were sown in mixed rows, although effects were not monitored beyond the establishment year. The approach is used occasionally in the establishment of pasture mixtures, where cover crops are not used (Wolfe and Southwood 1980, Sleugh *et al.* 2000, Butler *et al.* 2011) and is increasingly being used in the emerging field of perennial crop research, where legumes are grown in binary mixtures with perennial cereals (Hayes *et al.* 2017, Dick *et al.* 2018, Tautges *et al.* 2018).

At first glance, spatial separation at sowing offers significant potential. The practice is immediately applicable to commercial farms, likely to cause very little disruption to existing enterprise mixes and at little if any additional cost. Changing row configuration represents the same seed being sown on the same land but in a slightly different spatial arrangement, which would require only small modification to modern commercial seeders. In addition, changing row configuration presents a new opportunity to incorporate precision agriculture technologies into pasture systems. For example, where grass and legume species are sown in alternate rows, it may be possible to control annual grass weeds on just the legume drill row using selective herbicides. Under present management, very few grass-selective herbicides

can be used on newly-sown grass-based pastures (Dear *et al.* 2006). Alternatively, it may be possible to apply less phosphorus (P) fertiliser to the grass row than to the legume row to achieve optimal soil fertility for each pasture component. Current practice sees fertiliser applied across the entire landscape to a level that avoids deficiency in the legume component, but which is surplus to the requirements by the companion grass for optimal production (Simpson *et al.* 2014). Also, despite drill rows being sown in monocultures, the scale of spatial separation (defined by the row spacing of the particular sowing equipment) remains relatively small meaning that grazing livestock would still have access to a diverse diet and there could be increased interaction between species, such as nutrient cycling from excreta (Hilder 1966) or direct transfer of nutrients, compared to where species are sown in discrete plantings in adjacent fields and grazed in common (Pembleton *et al.* 2016).

This thesis examines the relative merits of separating species in different drill row configurations at sowing compared to the traditional approach, where multiple species are sown together in mixed drill rows. In the context of phased farming systems in rain-fed semi-arid environments, improved pasture composition may not only lead to increased pasture productivity and persistence but also to positive impacts on subsequent crops grown in rotation with those pastures. To that end, it is important to not only study the initial responses following sowing, but to also observe any enduring legacy of the original drill row that may affect productivity throughout and beyond the pasture phase. The thesis begins with a review of literature to explore the concept from a theoretical perspective, starting with some basic principles of plant competition. This is followed by five experimental chapters based upon two series of field experiments at multiple locations in southern and central New South Wales (NSW), Australia. The first cohort of experiments examine perennial and self-regenerating pasture species grown in an array of row configurations. Chapter 3 reports the differences in pasture biomass and composition observed at a network of five sites. Chapter 4 examines one site in detail to determine the residual effects of different drill rows on soil fertility and examines differences in yields of the following crop, measured at a fine spatial scale. Chapter 5 quantifies the legacy of the original pasture drill row on soil microbiology and seeks to understand key factors driving differences in the microbiome. The second series of experiments examines the effect of row configuration on pastures sown with a covercrop. The consequences of row configuration at sowing on lucerne survival is explored in some detail by combining data from both series of experiments in Chapter 6, as lucerne density is a major driver of productivity in lucerne-based swards (Palmer and Wynn-Williams 1976). Chapter 7 reports the crop and pasture biomass and composition data from the cover crop experiments,

and using data from both series of experiments, assesses the effects of changing drill row configuration in the transitions into and out of a pasture phase.

The objectives of the thesis are to i) determine the extent to which changing row configuration increases pasture and crop productivity in rain-fed semi-arid environments, ii) understand the mechanisms by which changing row configuration leads to crop and pasture responses and iii) utilise the results of the research to develop recommendations to inform land managers and their advisors if and when the use of alternative row configurations would likely lead to better production outcomes.

Chapter 2

Review of literature

This review of literature begins with a short description of the key pasture species important to pasture/crop rotations in southern Australia and by defining what is meant by a change in row configuration in the context of the body of work reported in this thesis. It will then explore some basic concepts of competition, particularly as it relates to the establishment of self-regenerating annual and perennial pasture species that are well adapted to the rain-fed, semi-arid environments of south-eastern Australia where they are commonly grown in phased rotations with annual winter crops. The final section examines key aspects of pasture succession beyond the establishment year to identify factors likely to be important to achieve a better balance of species throughout the life of the pasture.

Target species

The most productive and resilient pasture swards in semi-arid environments have been shown to be those pastures that contain species that are well-adapted to the target environment (Hayes et al. 2018). When grown in rotations with crops, the pasture species are expected to persist for the full pasture phase, which might typically be 3-6 years in southern Australia (Humphries and Auricht 2001). There are relatively few viable species options available to farmers in these semi-arid environments, particularly for use in phased rotations with crops (Hayes et al. 2012). Lucerne (alfalfa; *Medicago sativa* L.) is perhaps the only viable perennial legume option available, with all other perennial legume species shown to be substantially less productive and/or persistent over a range of soil types and climatic conditions across southern Australia (Li et al. 2008). Lucerne is estimated to be cultivated on 3.2 million ha across southern Australia, which is approximately half of the total area suggested as a realistic upper limit for adoption across that region (Robertson 2006). Phalaris (hardinggrass; *Phalaris aquatica* L.) is one of the four temperate perennial grass species most cultivated across southern Australia, along with cocksfoot (orchardgrass; *Dactylis glomerata* L.), perennial ryegrass (*Lolium perenne* L.) and tall fescue (*Festuca arundinacea* Schreb. = syn. *Lolium arundinaceum* (Schreb.) Darbysh.) (Oram and Lodge 2003). Of those species, phalaris is recognised as the most drought-tolerant, and therefore most broadly adapted, extending to regions receiving as little as 550 mm rainfall annually (Hill 1996). In fairness, that assessment of the drought hardiness of the four species possibly overlooks the highly drought tolerant *hispanica* subspecies of cocksfoot, which is known to occur naturally in substantially drier

environments, receiving as little as 270 mm annually (Oram 1990). Nevertheless, the drought hardiness and broad adaptation of phalaris is not in dispute and represents perhaps the best temperate perennial grass option available for use in drier cropping zone environments (Dear et al. 2004). Subterranean clover (*Trifolium subterraneum* L.) is the most developed annual legume, with over 45 registered cultivars adapted to a range of environments across southern Australia (Nichols et al. 2013). This species is broadly adapted to mixed (crop/livestock) farming and permanent pasture regions alike due to its high tolerance of uncontrolled grazing and acidic soils (Nichols et al. 2012). There has been increased interest in alternative pasture legume species for use in cropping environments in recent years (Nichols et al. 2007) but still none match the scale of adoption of subterranean clover.

Each of these species, although persistent and highly productive, pose significant risk of mortality in grazing livestock. Bloat is a risk in both subterranean clover and in lucerne (Brightling 1994), and red-gut is an additional risk to sheep in lucerne pastures (Gumbrell 1997). Risk of both conditions is elevated during winter and early spring when forage is highly digestible. Two other conditions, mainly in sheep, can be caused by phalaris, phalaris staggers and sudden death poisoning (Oram et al. 2009). Phalaris staggers can be prevented by supplementing the diet with cobalt, which is thought to stimulate bacteria in the rumen to break down the toxin. However, sudden death poisoning cannot be prevented other than with management of intake of fresh phalaris forage, usually in early autumn, which is thought to have cyanide and/or nitrate-based compounds that can cause mortality in sheep very soon after they begin grazing the pasture (Brightling 1994, Oram et al. 2009).

The widespread adoption of each of these species by farmers over many decades, despite the health risks they pose, is evidence that farmers perceive the advantages of incorporating them into their farming system to far outweigh the risks. It also highlights a widespread belief that those risks can be managed. The risks do not generally exist year-round but are confined to certain periods of the year, providing farmers some opportunity to destock paddocks at those times if other forage sources are available. However, in each species, the risk to livestock health is most easily managed when there is a diversity of forage available rather than exposing livestock to a single-species diet at those high-risk times (Brightling 1994, Oram et al. 2009). This speaks to the importance of finding management strategies to reliably establish and maintain a diverse pasture sward.

Definition of the change in row configuration

There are innumerable ways that row configuration could be changed at sowing. It could be as simple as changing row spacing, or changing the tyne assembly, or altering the geometry of the planting such as with two perpendicular passes, or even varying the time of sowing of individual sward components. For this thesis, row configuration refers to the spatial separation of species in equally spaced drill rows, with all components planted at the same time. The reason for focusing on this change of configuration is that it is the cheapest and most practical option, therefore the most likely to be adopted by farmers. More complex configurations that include multiple passes of the seeder, either to achieve temporal separation or to deliver perpendicular drill rows, represent a substantial additional cost in time and complexity and an increased barrier to adoption. In its simplest form, separating species in a binary mixture could mean sowing each species in alternate drill rows (a 1:1 configuration). It is prudent to examine the simplest options first as these will likely achieve greater industry impact if proven successful.

Seeding density due to the change of row configuration

There are two most prospective approaches to setting seeding rates with alternate drill row configurations:

- i) The 'area' approach where seeding rates achieve the same initial density of a given species on an area basis as would be achieved with a conventional mixed sowing arrangement.
- ii) The 'row' approach where seeding rates are adjusted such that any given sowing row delivers the same amount of seed as would have been delivered of that one species in a mixed sowing arrangement.

For example, we consider a hypothetical binary mixture of lucerne assuming a seed size of 460 000 seeds/kg and sown at 2 kg/ha, and subterranean clover assuming a seed size of 176 000 seeds/kg and sown at 5 kg/ha (sowing rates suggested by Wolfe and Southwood 1980). Both species are sown in conventional drill rows at 250 mm spacings in mixed rows, meaning that seed of both species is planted in every row. Assuming a completely even delivery of seed it is calculated that 23 lucerne and 22 subterranean clover seeds are placed in every 1 m of drill row; a total of 45 seeds. The same mixture sown using an alternate row configuration on an area basis would see the exact same quantity of seed sown per hectare but each species would be sown in only half the number of drill rows, and the sowing rate of that species in

each row would be doubled. In this example, that equates to 46 lucerne seeds/m placed in every second row, and 44 subterranean clover seeds/m placed in every other drill row. The increase in seedling density of a species in one row is offset by the complete exclusion of seed from the other species, although in this example the numbers of each species do not match exactly. By contrast, the same mixture sown using the 'row' approach would see the sowing rate per area halved. The density of each species within each drill row would be equivalent to the density of the same species within a row of the mixed sowing arrangement, but total plant density would decline because of the exclusion of the other species in that row. That is, the lucerne rows in this example would receive 23 seeds/m and the subterranean clover rows would receive only 22 seeds/m, compared to the 45 seeds in total when both species are sown in the same drill row. Due to the negative effects of reduced plant density on herbage yield (Palmer and Wynn-Williams 1976; Volenec 1987), it would seem that using the area approach to determine seeding rates with changed row configuration is the most appropriate approach in an agricultural context, particularly where seeding density is sufficiently low as to not cause seedling mortality through intra-specific competition (Linhart 1976). For all subsequent discussion we assume that changed row configurations would be undertaken at seeding rates determined on an area basis, meaning that seeding density remains consistent for a given species regardless of the configuration used.

Evaluation of the change of row configuration

Despite several studies adopting some form of changed row configuration to establish mixed swards of lucerne and subterranean clover (Hall *et al.* 1985), lucerne, phalaris (*Phalaris aquatica* L.) and subterranean clover (FitzGerald 1979, Wolfe and Southwood 1980) or various other species (Sleugh *et al.* 2000, Butler *et al.* 2011), few studies have actually evaluated the technique as a means to achieving more resilient mixed swards. Boschma *et al.* (2010) postulated that the establishment of mixed pasture swards in monoculture rows may enhance the establishment of C₄ perennial grass-based pastures by reducing the negative effects of interspecific competition from highly competitive species such as lucerne. There are several examples of the alternate row configuration being used where grain and pasture species are sown together, usually in a cover cropping context (McCormick *et al.* 2014). Perennial grass survival was enhanced when sown in alternate rows with a winter cereal compared to mixed row plantings (Hoen and Oram 1967, McCormick *et al.* 2014). Santhirasegaram and Black (1967) reported an increase in pasture production at the end of the establishment year in an alternate sowing configuration with wheat (*Triticum aestivum* L.) compared to mixed rows,

although only follow pasture yields for 15 months after sowing. In a lower rainfall environment, Scott (1985) observed no difference in annual medic forage production due to row arrangement conceding the result may have been confounded by the placement of fertiliser, which was only in the drill row of the crop. An increasing number of field studies evaluating novel perennial crop material with legumes are establishing experiments by spatially separating the crop and legume components (Dick *et al.* 2018, Tautges *et al.* 2018). Only one study has evaluated perennial crop performance when sown in different row configurations, in Cowra, Australia (Hayes *et al.* 2017). This experiment showed that separating the perennial wheat crop from the self-regenerating subterranean clover at sowing increased legume biomass by up to 128% but halved grain yields in year 2. In this experiment subterranean clover was unable to coexist at any meaningful level when planted in the same drill row as a vigorous perennial crop.

Competition for resources

To understand why subterranean clover benefitted from spatial separation from the perennial crop in the study by Hayes *et al.* (2017), it is important to revisit the basic principles of competition, particularly as it relates to the species in question. Clements *et al.* (1929) defined competition as it applies in the modern agricultural context: “when the immediate supply of a single necessary factor falls below the combined demands of the plants, competition begins”. Competition for light, water, nutrients and biological space exists between species (interspecific), between plants within a species (intraspecific) and between components of the same plant (intraplant).

Light competition - ceiling yield

As stated by Tow and Lazenby (2001) “the principles of competition and succession are directly relevant to the challenge of maintaining desirable pasture composition, free of weeds, while achieving a high level of productivity and utilisation”. Seedlings in a newly-sown sward that are small are often not in competition for resources as demand is low but as they develop, competition increases. Thus, the density of plants required to give maximum yields decreases as growth proceeds. The ultimate capacity of a species to produce biomass depends on the degree to which a community of such plants can exploit the light falling upon it. There exists a negative correlation in monocultures by which individual plant size decreases as density increases because in environments where water and nutrients are not limiting, full canopy cover cannot be exceeded to increase the utilisation of light. However, the large

plasticity of plants and the 'ceiling yield' imposed by available light dictates that maximum biomass production can be achieved at a very wide range of plant densities in some species. Donald (1951) demonstrated that maximum forage yield for a pure subterranean clover sward was achieved at 296 plants/m² but yield did not change as density increased 20-fold to 6167 plants. Whilst actual numbers may not be transferrable to different environments, the principle demonstrates the relative lack of importance of plant density in order to achieve maximum yield in this species, other than at very early stages of development. It is suggested that more plants may be needed to achieve full exploitation of a more favourable environment than to fully exploit a less favourable environment (Donald 1951). In environments where resources are more limiting, self-thinning of a population may occur.

Resource competition - self-thinning

Self-thinning is an important self-regulatory mechanism necessary for an overcrowded population (Yoda *et al.* 1963). An overcrowded population is unable to reach sound maturity or set sufficient seeds unless self-thinning occurs. Self-thinning is a density-dependant mechanism (Koyama and Kira 1956) and Yoda *et al.* (1963) describe a mathematical relationship they term the '3/2th power law' by which the herbage yield of plants (on an area basis) increases despite a reduction in plant density. An important question then arises in relation to the self-thinning phenomenon, specifically, which seedlings are thinned? Ross and Harper (1972) describe a dominance hierarchy by which early emerging seedlings dominate later emerging seedlings, providing a competitive advantage for the capture of light (Wilson 1988). Factors such as individual seed weights and early growth rates impact seedling dominance to only a minor extent, and other factors that might be supposed to impact relative competitive ability in the field, such as spatial variability of soil or heterogeneity were not considered by Ross and Harper (1972).

Self-regenerating annual species

It is important to distinguish competition in pure swards of annual species and contrast the dynamics with a mixture of annual and perennial species. In a newly sown annual-only sward such as described in Donald (1951;1954), seed is sown into a prepared seedbed in autumn. No competition exists initially, as the demand for resources by small seedlings is low, and resources are typically abundant. Following Clementsian theory, seedlings emerge and grow representing an increase in resource demand, and a reduction in resource supply soon follows (Clements *et al.* 1929). As growth accelerates at an exponential rate in spring, so too does the

intensity of competition for resources. Development of annual plants rapidly changes to form reproductive structures in preparedness for the inevitable depletion of resources. The annual plant would typically senesce following seed maturation with the lifecycle repeating itself the following autumn, although in the case of a self-regenerating species such as subterranean clover, germination in year 2 and subsequent years would be in the absence of a prepared seedbed but in the presence of established, mature perennial plants. However, a robust seedbank would ensure the emergence of a larger number of individuals the following autumn compared to that in the establishment year, enhancing the capacity for that generation of seedlings to utilise resources quickly. For example, recommended commercial sowing rates of subterranean clover typically do not exceed 10 kg/ha of seed (Lattimore and McCormick 2012), a fraction of the seed reserves that might exist in the soil. Seed reserves of 300-700 kg/ha has previously been estimated as being required to achieve robust regeneration of subterranean clover (Dear *et al.* 1993, Dear *et al.* 2001, Wolfe *et al.* 2006). These estimates undoubtedly allow for a proportion of non-viable and impermeable seed that will not emerge upon the first rains, but also reflect the need for greater seedling density when regenerating in an established pasture sward (Dear *et al.* 2007a).

Perennial species

Perennial species such as phalaris or lucerne rarely recruit from seed (Lodge 1991, Thapa *et al.* 2012) and so plant longevity is of paramount importance. Population dynamics in an establishing sward of a perennial species would be expected to be similar to that of an annual sward up until the first summer. The seed would be sown into a prepared seedbed and competition would begin when seedling growth rates and their demand for resources increased. The intensification of competition for resources in spring would also stimulate changes in perennial plant physiology and the formation of reproductive structures. However, unlike the annual plant, which would senesce following seed formation before repeating the life-cycle the following year, the perennial phalaris or lucerne plant is not reliant upon reproduction for persistence and therefore needs to survive the hot dry summer period. Water resources over summer are typically limiting in rain-fed semi-arid environments. For example, in a field evaluation of temperate perennial grasses in cropping environments of south-eastern Australia, Culvenor *et al.* (2016) calculated moisture deficit over summer to be approaching 540 mm at some sites in some years, a value well beyond the total rainfall that might be expected in a full year at the same sites. We might therefore expect competition for water to be intense over summer, a unique aspect of competition in perennial-based systems

compared to winter-growing annual species, which avoid the intense competition for water in summer as seed (Turner 1986, Ludlow 1989).

There are several mechanisms by which populations of perennial pasture plants compete for water during the summer period. The first is by developing survival structures that enable them to compete more effectively for a scarce resource. In the case of both phalaris and lucerne, the development of roots that penetrate deep into the soil profile, well beyond the depth that most annual plants can access, is key to their survival in water-limited environments (McWilliam and Kramer 1968, Christian 1977). Secondly, perennial plants can reduce demand through changes in growth patterns. In the case of phalaris, this is achieved through reduced tissue growth, herbage senescence and an incomplete dormancy mechanism, which in some situations precludes the plant from actively growing despite the temporary availability of water (Norton *et al.* 2012, Culvenor *et al.* 2017). Herbage senescence or 'leaf-drop' is also an important strategy for lucerne although in contrast to phalaris, there is no summer dormancy mechanism. Lucerne possesses genetic adaptations, which limit herbage growth where access to soil water is confined to deeper zones of the soil profile (Hoffmann *et al.* 2003), apparently conserving deep soil water for survival purposes only. Not all perennial forage species possess these water-conserving traits. White clover (*Trifolium repens* L.), for example, has limited capacity to conserve water (Norton *et al.* 2021) and limited access to deep soil water on account of its shallow root system (Caradus 1990). As a consequence, it is renowned as a species that is sensitive to drought (Lane *et al.* 2000).

The density of perennial plants inevitably declines over the first summer, even in species with adaptive dehydration tolerance and avoidance traits. Undoubtedly, the decline in density is strongly influenced by the level of intra-specific competition as previous studies have shown perennial plant density decline to be negatively correlated with initial plant density. Only at very low initial densities was a decline in density over the first summer not recorded (Palmer and Wynn-Williams 1976, Dear *et al.* 2007b). The density of various temperate perennial species has been shown to decline by up to 95% in rain-fed semi-arid environments, generally to around 15-40 plants/m², from initial emergence densities of in excess of 300 seedlings/m² (Hayes *et al.* 2010a, 2016). It is not clear when perennial plant mortality occurs over summer, due in part to the difficulties associated with measuring perennial plant mortality *in situ*. Firstly, it can be difficult to distinguish individual mature plants using non-destructive methods (Lodge and Gleeson 1984), particularly if the plant is dormant or in a state of reduced herbage growth. Secondly, particularly in species which display summer dormancy mechanisms, mortality is generally only observed retrospectively after the summer drought has broken and

the plant has failed to respond to rainfall (Norton *et al.* 2008). It is therefore difficult to ascribe plant mortality to competition or simply a sensitivity to edaphic factors, such as heat. In a field experiment where 95% of a tall fescue population apparently dies (Hayes *et al.* 2010a), it is prudent to question what were the specific attributes of the 19 remaining plants from the initial density of 370 plants/m² that enabled them to survive? It is of course impossible to know for certain, but we assume that intra-specific genetic or phenotypic differences exist that give some individuals within a population a competitive advantage over others (Nurjaya and Tow 2001). We might also expect that genotype interacts with the inherent spatial variability in soil characteristics (Conyers and Davey 1990, Conyers *et al.* 2018) and other characteristics of the micro-environment such that all plants, even from within the same genetic background, are not 'competing equally'.

There have been relatively few investigations to understand the competition dynamics of phalaris and lucerne grown in the same sward. Wolfe and Southwood (1980) grew lucerne at varying densities with either subterranean clover or phalaris by altering the row spacing of lucerne from 175 mm (lucerne in every row) to 525 mm (lucerne in every third row). Whilst the lucerne was never planted in drill rows alone in that experiment, the companion species, phalaris or subterranean clover, were planted in monoculture rows wherever lucerne was absent. That experiment demonstrated that the phalaris PX 18 genotype (a progenitor breeding line of cultivar Sirolan (Oram and Schroeder 1992)) was more productive when grown in mixtures with lucerne than the more summer active Siro 1146 (a similar genotype to the modern cultivar Advanced AT (R. A. Culvenor pers. comm.)). A later study demonstrated that phalaris genotypes that are earlier maturing and with higher levels of summer dormancy were more persistent under summer drought conditions than later maturing/lower summer dormancy types (Culvenor *et al.* 2017). Whilst the Wolfe and Southwood experiment did not set out to evaluate different row configurations or the persistence of the phalaris component, lucerne mortality was highest where it was grown with the summer active phalaris genotype suggesting that competition for water over summer was a major limitation of this interspecific relationship. When lucerne was grown in mixtures with phalaris or cocksfoot (*Dactylis glomerata* L.) cultivars with contrasting seasonal growth patterns, there was no consistent benefit in production and persistence associated with seasonal growth patterns (Hayes *et al.* 2018). Rather, the relative adaptation of a given cultivar to the particular environment was critical in determining the response of the sward.

In the context of interspecific competition, there are ramifications for reduced perennial plant density following the first summer. Most significantly, there are fewer individuals remaining to

utilise resources early in the autumn of year 2. As described previously, the ceiling yield imposed on any competing population might be achieved once there is full light interception, that is, at full canopy cover (Donald 1963). It is questionable whether full canopy cover can be achieved in pasture species at densities of only 15-40 plants/m². Palmer and Wynn-Williams (1976) showed in a series of field experiments on the Canterbury Plains of New Zealand that lucerne yields were depressed where lucerne density was very low (less than 30 plants/m²). They also illustrated the importance of seasonality of yields as swards with low lucerne densities yielded more over summer than more dense swards, suggesting that intraspecific competition was limited more by water than by light at that time of the year. It is acknowledged that Donald (1951) observed in a pure subterranean clover sward that initial plant density was relatively unimportant in the ability of the population to reach full canopy cover due to the plasticity of the plant community. However, those studies were considering populations with densities of 296-6167 plants/m²; well in excess of the numbers of perennial plants likely to survive a typical summer under dryland conditions, even in more temperate environments (Suzuki 1991).

Plasticity exists in communities of perennial pasture plants as it does in annual species but this varies with species. For example, phalaris spreads through the development of new tillers (McWilliam 1968) providing increased herbage growth. The study described above from New Zealand which observed increased herbage growth over summer in sparse lucerne stands compared to more dense stands is an example of plasticity in that species (Palmer and Wynn-Williams 1976). In drier environments with high summer temperatures, lucerne production did not decline until plant density fell to below 8 plants/m² (Bowman *et al.* 2002). Maximum ceiling yield is approached when full canopy cover is attained, and again, it would seem unlikely that this could be achieved at a density of 8 lucerne plants/m² regardless of how long the sward was left to develop. Therefore, it seems that low plant density beyond the establishment year is a constraint to the productivity potential of pure perennial pasture swards. Achieving the ceiling yield would be even more challenging in a perennial pasture sward that is periodically defoliated. Dear *et al.* (1998) showed that the amount of light reaching emerging clover seedlings doubled in defoliated phalaris swards compared to undefoliated swards, indicating the substantial impact defoliation has on the ability of a perennial to capture light. At the relatively low densities commonly reported in field experiments under rain-fed conditions, it seems highly likely that populations of perennial plants would often underutilise the available light.

Herein we identify an apparent conundrum: perennial plant density is reduced over summer by a constraint in available soil water to levels too low to achieve full canopy cover in subsequent years. It therefore seems impossible for pure perennial swards to achieve anything close to full production potential, particularly in favourable seasons for growth. It also seems likely that the insufficient plasticity of a pure perennial sward at low density increases the vulnerability of the pasture to invasion of weeds. Dear *et al.* (2007b) showed a negative curvilinear relationship between the invasion of a summer grass weed and density of lucerne or phalaris plants. Palmer and Wynn-Williams (1976) also showed weed incursion to increase in lucerne swards where density was low. For these reasons, a productive annual component is an essential feature of a mixed pasture sward in semi-arid environments, taking the emphasis from pure 'perennial' swards, as is much quoted in the literature, to 'perennial-based' swards. The perennial-based sward provides the theoretical benefits of increased stability offered by the perennial component coupled with the productivity offered by the annual species when conditions are favourable for growth.

Pasture sward diversity

Maintaining the balance

The challenges of managing a mixed sward, such as developing a grazing regime or a weed management strategy that does not bias one component or other require further elaboration. At the heart of this issue lies the question of whether the reported lack of success of achieving robust and stable mixtures of pasture species was an issue of establishment or an issue of ongoing management. There is little merit in exploring alternative establishment methods if the core of the problem is in the subsequent management of those pastures. Whilst it is not possible to go back to those previously quoted pasture surveys and ascertain the cause of failure of sward components, it is worthwhile examining the extent to which pasture management may impact results and to identify issues of management that may require particular attention if sowing configuration was changed.

Dear *et al.* (2007a) describe in detail the competitive relationships between subterranean clover grown with either lucerne or phalaris at two sites in southern NSW, Australia. Clover seedling regeneration in autumn was positively related to the quantum of clover seed reserves in the soil during summer but inversely correlated to phalaris and lucerne density. This was partly because clover seed yields were inversely related to perennial plant density, but phalaris was shown to depress clover seed yield more than lucerne. The authors ascribed

this result to the increased shading from phalaris of subterranean clover during seed set compared with the lucerne swards (Dear *et al.* 2000) reflective of the inherent sensitivity of subterranean to shading (Stern and Donald 1962). So, subterranean clover regeneration would be reduced by the presence of a perennial species which, in the case of phalaris particularly, could depress seed set through competition for light in spring reducing the size of the clover seedbank over summer and therefore the regenerative capacity of the clover the following year. Grazing the phalaris/subterranean clover sward in spring may be one avenue to reduce the competition for light and maximise seed set, due to the positive response of defoliation on subterranean clover seed yield (Collins 1978). However, depending upon the severity of grazing and the phalaris cultivar used, this may reduce phalaris persistence (Culvenor 1994), tipping the competitive balance in favour of subterranean clover.

Although light interception governs the 'ceiling yield' of a pasture sward, water deficit is overwhelmingly the key limiting factor to pasture forage production in rain-fed, semi-arid environments. Lucerne and phalaris are both deep-rooted perennial species with reputations for a superior capacity to dry the soil profile to depth (Lolicato 2000, Hayes *et al.* 2010b) but here we identify a second conundrum: We are proposing to design pasture mixtures which make greater use of the available light resource by combining the stable influence of the perennial component with the highly productive and plastic characteristics of the annual component. However, we seek to do this in an overwhelmingly moisture-limiting context and by adding perennials to the mix, we are further elevating the moisture stress on the annual component of the sward. This effect was illustrated by Dear *et al.* (1998, 2007a) who showed that survival of subterranean clover seedlings was inhibited by the presence of a companion perennial species compared to where subterranean clover was grown as a monoculture. The consequence of this elevated moisture stress can be substantial in a species such as subterranean clover which is known to be highly sensitive to water stress at the seedling stage (Roberts 1966, Dowling *et al.* 1971). McWilliam *et al.* (1970) showed that subterranean clover germination was negatively affected at an osmotic potential of -250 kPa, compared to annual ryegrass (*Lolium rigidum* Gaud.) which appeared unaffected at -800 kPa. There is little evidence that seedlings of alternative annual legume species would be any less sensitive to early moisture deficits than subterranean clover, although with a greater range of species and cultivars now available (Nichols *et al.* 2012) this would seem to be a fruitful line of further investigation.

The persistence of the annual legume is reliant on its ability to maintain a sufficient seed bank. Wolfe *et al.* (2006) suggested a minimum seedbank for subterranean clover pastures of 600

kg/ha in summer and 300 kg/ha in winter, not dissimilar to the 700 and 500 kg/ha benchmarks for summer and winter, respectively, proposed by Dear *et al.* (1993, 2001) for a similar environment. Wolfe and Southwood (1980) recommended that the planting of earlier maturing cultivars would enhance the persistence of subterranean clover grown in mixed perennial-based swards. Dear *et al.* (2007a) tested this hypothesis reasoning that earlier maturing cultivars could reduce the intensity of competition with perennial species in spring. However, this hypothesis was rejected because there was little evidence that the inclusion of perennial species hastened the onset of moisture stress in spring. A subterranean clover cultivar that was shown to be more tolerant to moisture stress at the seedling stage or that was more tolerant of shading in spring could be at an advantage in mixtures with perennial species, but as yet little evidence exists that subterranean clover cultivars display a differential tolerance to these factors. Until such research is conducted it can be assumed that the competition between perennial species and subterranean clover will need to be reduced in order to maximise clover seed production, by reducing competition for water at the seedling stage and the competition for light during seed set in spring. Cultivar selection will not overcome this problem.

Changing competition dynamics

Most studies of seedling competition do not consider competition in terms of a linear row but rather in multi-dimensional concepts of biological space, relative to the position of a particular seedling. This is appropriate because seedlings intercept resources from all directions around them. The linear drill row is therefore an artificial construct imposed on agricultural plant communities for the purpose of human convenience. In cropping contexts where much recent innovation has occurred with seeding techniques to avoid complete cultivation of land and to retain stubble, it is acknowledged that the trend towards wider row spacing is to achieve mechanical advantage rather than to promote plant productivity, *per se* (Scott *et al.* 2013). Nevertheless, the linear row governs the initial placement of seeds and seedlings. Consequently it is an important consideration in understanding population dynamics of pasture communities.

Experiments conducted by Ross and Harper (1972) showed that the presence of neighbouring plants can interfere with resource capture by an individual seedling and that the individual seedling can respond to this interference by expanding in a direction of lesser interference. In a field context where seedlings are emerging from newly sown drill rows (and assuming background weeds are controlled through normal management), it might be supposed that

the concentration of seedlings within the row might soon cause seedling development to expand beyond (largely perpendicular to) the drill row to explore the inter-row space. Clearly, species with a more erect growth habit are at a disadvantage in this respect than more prostrate species. Where single species are sown in drill rows, we might assume that early interference is in the form of intra-specific competition, and that the intensity of intra-specific competition is positively correlated with seeding rate and initial seedling density. Self-thinning may occur within the drill row in instances where seedling density is too great or the resources too limiting (White and Harper 1970), although few empirical studies have ventured to quantify maximum thresholds of phalaris, lucerne or subterranean clover that can exist within a drill row in rain-fed semi-arid environments before self-thinning is likely to occur. In a study of wheat, Amjad and Anderson (2006) showed that self-thinning began to occur at densities greater than 150 plants/m² in a Western Australian wheatbelt environment, presumed to be a consequence of intraspecific/intra-row competition. By contrast, density-dependant germination whereby seedling germination is enhanced by greater density has been observed in a small number of species and is attributed either to increased moisture levels associated with groups of closely packed seeds or chemical growth factors released by germinating seeds (Linhart 1976, Waite and Hutchings 1978). There is little evidence of such effects reported for lucerne, phalaris or subterranean clover. The experience of Donald (1951) with subterranean clover might suggest that self-thinning is unlikely to occur to any great extent due to the plasticity of that species, and that any self-thinning which might occur would be overwhelmingly governed by rainfall and other site factors.

Row spacing is an important consideration because at wide row spacings, intraspecific competition is more intense due to a greater density of seedlings within fewer drill rows. By contrast, a narrower row spacing theoretically subjects individuals to interspecific competition earlier due to the reduced distance between competing species in alternate drill rows. The distance between sowing rows is arbitrary and dependent upon individual farmers and the machinery that is used. In practice, it is unlikely to find seeding rows spaced narrower than 150 mm due to physical limitations of machinery (Scott *et al.* 2013). However, it is possible to have an arrangement where seed comes down either side of each tyne leaving a smaller distance between two rows and a larger distance between tynes (so called 'ribbon' or 'paired rows'), or use scatter plates and other arrangements to increase the spread of seed within a row to as much as 75 mm (Amjad and Anderson 2006). There is an almost infinite array of tyne assemblies possible that might influence row spacing. The trend towards wider rows in cropping enterprises to better accommodate crop stubble has seen row spacing increase to as

much as 420 mm (Scott *et al.* 2013). Pasture row spacings are traditionally much narrower, often around 180 mm (Santhirasegaram and Black 1967). However, with the trend towards wider row spacing in crops it seems inevitable that row spacing of pastures also increases where they are grown in rotation with crops. This is primarily because the same machinery is often used on a given farm to sow both crops and pastures, and the need to manage stubble loads can be as important when sowing a pasture as it is when sowing the next crop. Therefore, we might assume that row spacings of 150-300 mm are common in conventional farming systems, in south-eastern Australia at least.

Moving beyond the drill row

Santhirasegaram and Black (1967) showed that pasture biomass in the establishment year was greater when a cover crop of wheat was planted in rows approximately 180 mm from the pasture row compared to where it was in the same row, inferring that physical separation reduced interspecific competition between species early in their development. For regenerating seedlings in established swards, inter-specific relationships can be quite complex (Tow and Lazenby 2001) and at certain times of the year there may be advantages for an emerging seedling to be located close to the base of an established perennial plant. Over summer the crown of the perennial plant may intercept seed pods moved by water, wind or livestock. It may also provide a favourable micro-climate for the emerging seedling in autumn, creating a soil environment more conducive to germination and emergence, and shading the young seedling from intense heat or wind. However, close proximity in autumn exposes the seedling to intense competition for light, water and nutrients (Dear and Cocks 1997) in which the seedling will almost always be found vulnerable.

The movement of a species beyond the drill row is clearly dependent upon the physiology and habit of that species as it interacts with its environment. Lucerne has a semi-erect growth habit and is therefore limited in its ability to spread far beyond the initial place of emergence. Notwithstanding, lucerne genotypes have the capacity to increase the number of shoots per plant (Pembleton *et al.* 2010), subject to the availability of resources, which over time serves to increase the size of the lucerne crown at the soil surface. However, it seems unlikely that lucerne crowns will encroach on neighbouring rows where conventional row spacing (≥ 150 mm) is used. Phalaris by contrast has a more prostrate growth habit, although this can vary between cultivars. The persistence of phalaris plants is strongly related to the formation of dormant buds that appear at the base of old tillers, usually just below the soil surface, from which new tillers ultimately develop (Culvenor 1993). Under favourable growing conditions

phalaris plants can spread over a relatively large (dinner-plate size) area with large numbers of dormant buds and through subsequent tillering. It may be possible for mature phalaris plants to begin to encroach on neighbouring rows after several years of growth under favourable conditions.

Being an annual plant, the movement of subterranean clover is far less predictable.

Persistence of that species is reliant upon regeneration from seed which, as described previously, is highly correlated with the size of the seedbank (Rossiter 1966, Dear *et al.* 2007a). The location of the seed is determined by its initial placement by the maternal parent, and any displacement occurring between seed set and germination attributable to wind, water, soil subsidence or the actions of fauna, humans and grazing livestock. Initial placement of seed is governed by the size of the maternal plant, that is, the distance of reproductive structures from the crown (Donald 1954) as well as soil conditions during seed set.

Regeneration is more successful from buried rather than unburied pod, and typically a greater proportion of buried pod are those formed earlier due to more moist soil conditions in early spring (Yates 1957). Earlier-formed pods will, by definition, exist closer to the crown of the maternal plant than later-formed pods. Runner length may be further reduced in grazed swards due to defoliation but realistically this may have a limited impact on ultimate seed placement due to the prostrate habit of subterranean clover (Gladstones 1966) and the increased number of inflorescences per unit area observed in grazed subterranean clover swards compared to ungrazed swards (Rossiter 1961). The creation of drill rows at sowing modifies the microenvironment near the plant, bringing water to the base where seed was originally placed. In addition, detached plant material that might act as a mulch at the micro scale is more likely found around the base of plants which are more concentrated in rows early in the life of a pasture sward. It is therefore reasonable to assume that the soil surface is more likely to be slightly wetter in the drill row than between rows. Given the importance of moisture at the soil surface for viable seed production (Yates 1957), and given the importance of early emergence in the previously described dominance hierarchy (Ross and Harper 1972) one might postulate that the occurrence of viable regenerating seedlings is more probable close to the original sowing row than the inter-row area. More research is required to test this hypothesis, as well as to determine how long the effect of the micro-environment (caused by the creation of the drill row) might last.

Conclusions

Based on the evidence available, and in the absence of many prior studies that have tested the practice directly, further experimentation in changing drill row configurations at sowing is warranted. It seems likely that some sort of intervention is necessary to help manage the interspecific competition that exists in mixed swards. A pilot study provides some confidence that the practice has merit, demonstrating that adjusting row configuration to separate species assisted in managing the competition between subterranean clover and early-generation perennial wheat derivatives (Hayes *et al.* 2017). Restricting perennial species to fewer drill rows would seem to be less of an issue in rain-fed semi-arid environments as it might be in more favourable climates due to the plasticity of plants and their ability to compensate for yield, even at very low plant densities but this assumption requires field validation. Another underlying assumption is that sward components can move beyond the drill row after the establishment year to explore the light and other resources available in the inter-row area. The self-regenerating annual legume, subterranean clover, would seem well placed to perform this function over time in a perennial-based sward.

A large part of the appeal of this practice is its relative simplicity for farmers. The fact that farmers would be putting the same seed on the same area of land without alteration to the broader rotation would make this intervention seem to be highly adoptable on account of the very minor increases in cost and management complexity that such a change might require. However, so that a farmer can fully assess the merits of this practice it is necessary to explore any legacy effect that changing row configuration may have on the cropping phase of the rotation. If changing row configuration is proven successful in increasing pasture productivity and legume composition, it is likely that there will potentially be flow-on benefits to crops due to factors such as increased N inputs from the legume components of the pasture given the close relationship between N-fixation and legume productivity (Peoples *et al.* 2012). Again, this requires testing in the field.

The following chapters test the effects of changing row configuration in two series of field experiments. The first series, conducted in the Riverina region of New South Wales (NSW), examines various combinations and row configurations of pasture mixtures that include perennial and self-regenerating annual species that are commonly used in pasture/crop rotations in this region. The first experimental chapter (Chapter 3) reports pasture yields and persistence across five contrasting sites. Chapter 4 examines one site in detail to understand the legacy of the pasture drill row on soil fertility and related to subsequent wheat yields

measured at a fine spatial scale. At that same site, an investigation of the diversity and abundance of micro-organisms associated with the pasture drill row was undertaken and is reported in Chapter 5. The second series of experiments was conducted in the Central West of NSW and included a more extreme contrast in plant growth habits compared to the first series, by examining the effects of changing row configuration when legume pastures were established with a cover crop. Chapter 6 uses data from both series of experiments in a detailed assessment of the consequences to lucerne persistence of changing row configuration. The final experimental chapter, Chapter 7, reports the crop and pasture biomass production achieved in the second series of experiments, and also assesses the residual benefits of changing row configuration in the transition back into a cropping phase using data from three sites from the Riverina region. Combined, this body of work enables the assessment of the difference in productivity attributable to changing row configuration in mixtures of pasture species as well as crop and pasture species, and the legacy of such effects beyond the year of sowing within a pasture phase and in the transitions between crop and pasture phases.

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Chapter 3

Enhancing composition and persistence of mixed pasture swards in southern New South Wales through alternative spatial configurations and improved legume performance

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Enhancing composition and persistence of mixed pasture swards in southern New South Wales through alternative spatial configurations and improved legume performance

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Abstract. This study examined whether the productivity and persistence of mixed pastures that included subterranean clover (*Trifolium subterraneum* L.), lucerne (*Medicago sativa* L.), and/or phalaris (*Phalaris aquatica* L.) were improved if species were spatially separated rather than being sown together in each drill row. The study also compared the productivity and persistence of subterranean clover with alternative annual legume species biserrula (*Biserrula pelecinus* L.) and strand medic (*Medicago littoralis* Rhode ex Loisel). Twelve pasture treatments were sown in replicated field experiments at five locations across the medium-rainfall region of south-western New South Wales in 2012 and monitored for 3 years. Pastures that included lucerne, phalaris and subterranean clover were generally more productive than pastures with only one or two of those species, regardless of sowing configuration. Averaged across sites, subterranean clover regeneration in year 3 was 29% higher and total cumulative biomass 13% higher where subterranean clover was sown in a 1 : 1 configuration with lucerne than where the species were mixed together in every drill row. There were fewer consistent benefits of alternative spatial configurations on swards containing phalaris with subterranean clover or with lucerne. Results of the present study appeared to be highly site-specific, or season-dependent, and therefore alternative spatial configurations cannot be recommended as a universal strategy at this time. More research is required to understand the factors driving the responses to alternative spatial configurations observed in the present study.

Neither biserrula nor strand medic was superior to subterranean clover in a 3-year pasture phase with lucerne, despite abundant rhizobia compatible with all species at all sites. The density of subterranean clover in year 3 was 29% and 41% higher than of biserrula and strand medic, respectively. A narrow choice of adapted cultivars as well as excessive levels of hard seed for the legumes used in phased pastures with lucerne are suggested as contributing to the inferior performance of the alternative legumes species tested in the study.

Additional keywords: alfalfa, annual pasture, nitrogen fixation, population ecology, seed production.

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Introduction

Agriculture in the medium-rainfall (average 450–600 mm) cropping region of south-eastern Australia commonly comprises integrated crop and livestock production with extensive pastures grown in phased rotations with winter grain and oilseed crops (Bell *et al.* 2014a, 2014b). Pastures in these environments are grown under rainfed conditions to support grazing livestock and to improve soil fertility for the subsequent cropping phase (Chan *et al.* 2001; Peoples *et al.* 2001). Pastures in this region are also required for ecosystem services such as mitigating the risk of soil degradation associated with deep drainage (Dunin and Passioura 2006; Sandral *et al.* 2006; Hayes *et al.* 2010b) as well as providing protective cover to the soil surface. Periodic

drought is common and therefore persistence over the duration of the pasture phase is a key measure of the potential contribution of pasture species in this region.

Perennial pasture species such as lucerne (alfalfa, *Medicago sativa* L.) and phalaris (*Phalaris aquatica* L.) generally persist through longevity. Chance events of seedling recruitment are relatively rare, due to unfavourable seasonal conditions as well as farmer management practices, which generally aim to accommodate the needs of livestock rather than optimise seed production in a grazed sward (Dear *et al.* 2007b; Thapa *et al.* 2011). Therefore, individual plants must endure periodic droughts by using a range of mechanisms including increased access to soil water through deep roots (McWilliam and Kramer

1968; Ward and Micin 2006) and traits that enable dormancy or inactivity during periods of severe moisture stress (McWilliam 1968; Christian 1977). Consequently, interspecific competition between perennial species in a water-limited environment can be very finely balanced, with the most aggressive and drought-tolerant perennial species often quickly dominating a more sensitive perennial species under periods of acute water deficit. Spatial separation of perennial species at sowing has been suggested as a means to reduce interspecific competition at the seedling stage (Harper 1977; Boschma *et al.* 2010). This is a strategy used with some success in the establishment of lucerne–tall fescue (*Festuca arundinacea* Schreb syn. *Lolium arundinaceum*) binary mixtures in generally drier environments across Texas and Oklahoma, USA (Butler *et al.* 2011). There are relatively few viable alternative perennial pasture species in the medium-rainfall cropping region of south-eastern Australia, with lucerne and phalaris consistently proving the most productive and persistent species available (Dear *et al.* 2001; Li *et al.* 2008; Hayes *et al.* 2010a). However, there are almost no published reports detailing their performance when grown together in mixed swards.

By contrast, regenerating annual species persist because of traits associated with seed production and seedling recruitment (Rossiter 1966). Annual pasture legumes have been fundamental to the integrated livestock production systems of south-eastern Australia on account of their productivity and forage quality for livestock production, as well as their capacity to fix large quantities of atmospheric dinitrogen (N₂) fundamental to crop and livestock performance (Dear *et al.* 2003; Nichols *et al.* 2012; Peoples *et al.* 2012). Yet few studies have assessed the performance of regenerating annual legume species when grown in mixtures with perennial species. Forage production, inputs of fixed N and persistence of subterranean clover (*Trifolium subterraneum* L.) were shown to decrease in the presence of lucerne or phalaris, due to increased competition for light, water and nutrients (Dear *et al.* 1998; Dear *et al.* 2000). However, planting subterranean clover in drill rows spatially separated from a perennial wheat crop increased subterranean clover persistence, most likely through the effect of competition for light and moisture on seed production by the vigorous crop canopy (Hayes *et al.* 2016).

The ability for different species to coexist within the same sward for an extended period will be driven, to some degree, by the competitive nature of the species in question. The assumption that subterranean clover remains the most productive self-regenerating annual legume option available in the target region has been challenged recently for several reasons. First, several decades of research and development have culminated

in the release of an unprecedented array of alternative species and cultivars with demonstrated adaptation to a range of environments and farming systems (Nichols *et al.* 2007). Second, the evolution of crop and livestock production systems has led to changes in factors such as stubble management, grazing intensity and cropping frequency, which may have undermined, to some extent, the ecological advantages subterranean clover traditionally enjoyed. Third, concern exists about the suspected impact of residual herbicides applied during the cropping phase on subterranean clover during the pasture phase. At least one cultivar of strand medic (*Medicago littoralis* Rhode ex Loisel) is now commercially available with demonstrated tolerance to sulfonyleurea herbicide residues commonly used in modern crop rotations (Nichols *et al.* 2007).

Indeed, the inclusion of perennial species in the pasture phase itself represents a substantial shift in the production system that is likely to disadvantage subterranean clover. Lucerne and phalaris increase the competition for water during autumn when the annual legume is regenerating, inducing additional ‘false breaks’ at the start of the growing season (Dear *et al.* 2007a). Annual legume species such as annual medics or new species such as biserrula (*Biserrula pelecinus* L.) with higher levels of hard seed (Nichols *et al.* 2007) may now offer an advantage with a greater proportion of seed reserves expected to remain dormant for longer, potentially reducing vulnerability to false breaks compared with the softer seeded subterranean clover.

The study explored three hypotheses: (i) that spatial separation of lucerne and phalaris will lead to a more balanced mixed sward under field conditions than when the species are sown together in every drill row; (ii) that spatial separation of subterranean clover from lucerne or phalaris will increase persistence of the annual legume compared with when both annual and perennial species are grown together in the same drill row; and (iii) that the herbage yield of subterranean clover when grown with lucerne is inferior to that of alternative annual legumes that are either harder seeded or more tolerant to residual herbicides.

Materials and methods

Site and treatment details

A series of field experiments was sown in May 2012 at five locations on red soils in the medium-rainfall cropping region of south-western New South Wales (NSW), near the townships of Mirrool (average annual rainfall 471 mm), Lockhart (484 mm), Wagga Wagga (530 mm), Eurongilly (535 mm) and Burrumbuttock (580 mm). Initial soil test data for each of the experimental sites are provided in Table 1. Twelve treatments were included to compare the establishment and succession

Table 1. Available phosphorus (P) and sulfur (S), cation exchange capacity (CEC), calcium : magnesium ratio (Ca : Mg), aluminium (Al) saturation and pH of the surface 0.1 m of the five experimental sites

Site	pH _{CaCl2}	Colwell P (mg/kg)	S (KCL ₄₀ , mg/kg)	CEC (cmol(+)/kg)	Ca : Mg	Al (%)
Mirrool	4.8	60.0	6.2	7.5	4.7	2.6
Lockhart	4.8	39.0	18.6	8.9	6.3	2.3
Wagga Wagga	5.1	51.9	18.8	8.3	8.0	1.0
Eurongilly	4.9	25.6 ^A	14.0	6.6	8.1	–
Burrumbuttock	4.6	38.5	30.2	5.4	6.5	5.2

^AOlsen P value.

from 2012 to 2014 of mixed swards sown to different pasture species combinations and sowing configurations (Table 2). The treatments were grouped into lucerne–subterranean clover (Group I), phalaris–lucerne with subterranean clover (Group II), and lucerne–annual legume (Group III), with the lucerne-only treatment common across all three groups. Multiple cultivars of lucerne and subterranean clover were sown in equal proportions by weight (Table 2), but only single cultivars of phalaris (cv. Sirolan), strand medic (sulfonylurea tolerant cv. Angel) and biserrula (cv. Casbah) were sown owing to the lack of adapted cultivars of these species in the target region. Sowing rates were selected according to guidelines developed for environments across NSW (Lattimore and McCormick 2012). The sowing rates of phalaris and lucerne were halved when mixed together to achieve a similar total perennial plant density, but the subterranean clover seeding rate was unchanged regardless of treatment.

Each plot was direct drilled with 16 rows of pastures at a row spacing of 250 mm by using an 8-tyne cone seeder with narrow points and press-wheels. The seeding rate of a particular species was kept the same on an area basis (kg/ha) regardless of row configuration. The alternate row (1 : 1) treatment was achieved by condensing the number of rows into which seed of a particular species was drilled from 16 to 8 rows/plot, resulting in a 100%

increase in seeding rate per drill-row. In the double skip-row treatments, such as lucerne–subterranean clover (1 : 2) where one row of lucerne was sown to every two rows of subterranean clover, the subterranean clover was condensed from 16 to 10 drill-rows/plot and the lucerne was condensed to the remaining 6 rows/plot. In this example, seeding rate per drill row was increased by 60% and 167% for subterranean clover and lucerne, respectively. In the treatments that contained a 3-way mixture of lucerne, phalaris and subterranean clover (phalaris–lucerne (1 : 1), phalaris–lucerne (1 : 2), phalaris–lucerne mix), subterranean clover was sown in equal proportions in every drill row together with the perennial species (Table 2). All experiments were randomised in a row and column design with three replicates. Plots were 12 m by 4 m at all sites except at Wagga Wagga where they were 6 m by 4 m.

Management of sites and seasonal conditions

All sites were on commercial farms except the Wagga Wagga site, which was on a research station. Broad-spectrum herbicides such as glyphosate were applied to all sites in the spring and summer before sowing to reduce weed burdens. Approximately 150 kg/ha of Starter 15 (14.7% N, 13% P, 12% S; Incitec Pivot Fertilisers, Melbourne) fertiliser was broadcast onto the soil surface immediately before sowing. At the Burrumbuttock and

Table 2. List of treatments sown at Mirrool, Lockhart, Wagga Wagga, Eurongilly and Burrumbuttock, NSW

Treatments grouped for comparison purposes. Subterranean clover cultivars varied depending on rainfall, with cvv. Gosse, Goulburn and Coolamon at high-rainfall sites (Burrumbuttock and Eurongilly), cvv. Riverina, Coolamon and Bindoon at medium-rainfall sites (Wagga Wagga and Lockhart), and cvv. Trikkala, Bindoon and Dalkeith at low-rainfall site (Mirrool)

Sowing mix (drill-row ratio)	Cultivar and sowing rate	Description
<i>Group I. Lucerne–subterranean clover</i>		
Lucerne only	Lucerne (50% Aurora, 50% Genesis) at 3 kg/ha	Lucerne sown in every drill-row
Lucerne–subterranean clover (1 : 1)	Lucerne (50% Aurora, 50% Genesis) at 3 kg/ha, subterranean clover at 4 kg/ha (3 cultivars, 1/3 each)	Lucerne and subterranean clover sown in alternate drill-rows
Lucerne–subterranean clover (1 : 2)	As above	Two rows of subterranean clover to every row of lucerne
Lucerne–subterranean clover mix	As above	Lucerne and subterranean clover sown together in every drill-row
Subterranean clover only	Subterranean clover at 4 kg/ha (3 cultivars, 1/3 each)	Subterranean clover sown in every drill-row
<i>Group II. Phalaris–lucerne with subterranean clover</i>		
Phalaris–subterranean clover mix	Sirolan phalaris at 3 kg/ha, subterranean clover at 4 kg/ha (3 cultivars, 1/3 each)	Phalaris and subterranean clover sown together in every drill-row
Phalaris–subterranean clover (1 : 1)	As above	Phalaris and subterranean clover in alternate drill-rows
Phalaris–lucerne (1 : 1) with subterranean clover	Sirolan phalaris at 1.5 kg/ha, lucerne (50% Aurora, 50% Genesis) at 1.5 kg/ha, mixed with subterranean clover at 4 kg/ha (3 cultivars, 1/3 each)	Phalaris and lucerne sown in alternate drill-rows; subterranean clover sown in every row
Phalaris–lucerne (1 : 2) with subterranean clover	As above	Two rows of lucerne to every row of phalaris; subterranean clover sown in every drill-row
Phalaris–lucerne mix with subterranean clover	As above	Phalaris, lucerne and subterranean clover sown in every drill-row
Lucerne only	As described in Group I	As described in Group I
<i>Group III. Lucerne–annual legume</i>		
Lucerne–biserrula (1 : 1)	Lucerne (50% Aurora, 50% Genesis) at 3 kg/ha, Casbah biserrula at 1 kg/ha	Lucerne and biserrula sown in alternate drill-rows
Lucerne–medic (1 : 1)	Lucerne (50% Aurora, 50% Genesis) at 3 kg/ha, Angel medic at 3 kg/ha	Lucerne and strand medic sown in alternate drill-rows
Lucerne–subterranean clover (1 : 1)	As described in Group I	As described in Group I
Lucerne only	As described in Group I	As described in Group I

Lockhart sites, omethoate (100 mL/ha at 290 g/L) was sprayed once at 2 months after sowing to reduce damage to emerging seedlings from insect pests such as redlegged earth mite (*Halotydeus destructor*). No other pesticides were applied to the experiments during the sampling period.

Fences were erected following sowing on all of the commercial farms to control grazing by livestock. Following assessments of aboveground biomass, sites were grazed by a large number of sheep for 1–2 days to remove biomass and avoid preferential grazing. On occasions when sheep were not available or were not able to graze the site evenly, the experimental area was mown. No grazing occurred at the Wagga Wagga site, but the site was mown with a forage harvester immediately following sampling, and herbage was removed.

All sites experienced substantially wetter than average seasonal conditions in the late summer–early autumn (February–March) before sowing in 2012 (Fig. 1). However, monthly rainfall following sowing at each site remained generally at or below the long-term average, with the experimental period being characterised by a series of dry springs (September–November).

Plant density and basal frequency

Seedling establishment density was determined ~12 weeks post-sowing by laying a quadrat 1.5 m by 0.5 m, with grids 0.1 m by 0.1 m, at two fixed locations per plot and counting the number of seedlings in each grid. Care was taken to lay the long edge of the quadrat perpendicular to the drill rows to ensure the appropriate number of drill-rows was counted each time. Initial basal frequency of lucerne and phalaris was determined at the same time by using the same quadrats at the fixed locations within each plot and counting the number of grids containing the stem base of a perennial seedling. Annual legume regeneration and basal frequency of perennial species were reassessed annually in autumn of years 2 and 3, except at Eurongilly where annual legume regeneration was not sampled in year 3.

Herbage biomass

Aboveground biomass was assessed visually at the end of spring in year 1, and at 3-monthly intervals thereafter at 10 locations across a transect of each plot. The visual scores were converted to dry matter (DM) by taking up to 25 calibration cuts per site at each sampling date. Botanical composition was assessed at

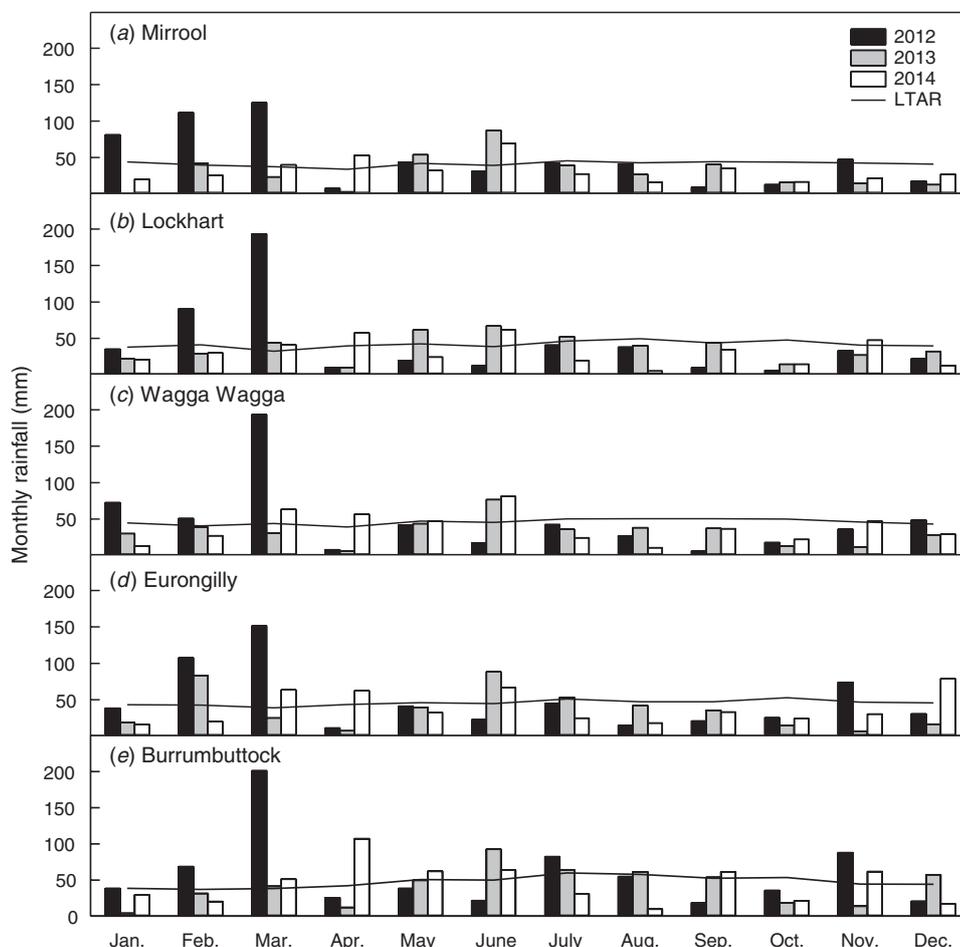


Fig. 1. Monthly rainfall (mm) in 2012–14 compared with the long-term average (LTAR) at (a) Mirrool, (b) Lockhart, (c) Wagga Wagga, (d) Eurongilly, and (e) Burrumbuttock, NSW.

10 locations on the same day by using the dry weight rank method ('t Mannetje and Haydock 1963). The herbage DM of each species was calculated from aboveground biomass multiplied by the proportion of each species in the swards. Cumulative biomass was calculated as the sum of biomass across all samplings over period of 3 years, a technique that provides a sound measure of relative performance but tends to overestimate productivity because it does not deduct the residual biomass remaining following defoliation of the site post-sampling.

Nitrogen fixation

Samples of herbage were retained from each plot during winter and spring each year for N analysis. Samples were analysed for N concentration (%N) and ^{15}N composition by using an automatic N and carbon analysis (ANCA-SL) interfaced to a 20–20 stable isotope mass spectrometer (Europa Scientific, Crewe, UK). The amounts of aboveground (shoot or herbage) N accumulated by the different treatments were calculated from measures of herbage DM and N concentrations as:

$$\text{Herbage N} = \text{herbage DM} \times (\%N/100)$$

The proportion of legume herbage N derived from N_2 fixation (%Ndfa) after the initial establishment year was calculated by using the ^{15}N natural abundance method based on the differences between the ^{15}N abundance (‰) of phalaris and the sown legume species, and the glasshouse-derived determinations of the ^{15}N composition of shoots of legumes grown with N_2 as the sole source of N (Unkovich *et al.* 2008). The ^{15}N abundance of phalaris-forage harvested from each experiment in late spring 2013 and 2014 ranged from 2.48‰ to 7.40‰ (mean 4.64‰ over all sites and years) and was assumed to provide a location-specific determination of the ^{15}N composition of plant-available soil N during the peak period of pasture DM production. The equivalent measures of ^{15}N abundance of lucerne (0.44–4.01‰, mean 2.07‰), subterranean clover (–0.22 to 2.61‰, mean 0.67‰), biserrula (0.15–2.63‰, mean 1.09‰), and strand medic (0.34–4.30‰, mean 1.91‰) were all lower than detected in phalaris and were indicative of the relative reliance of the different legume treatments upon N_2 fixation for growth.

The amounts of shoot N fixed were calculated from determinations of legume herbage N and derived estimates of %Ndfa as (Unkovich *et al.* 2008):

$$\text{Inputs of fixed N} = [\text{legume herbage N}] \times \%Ndfa/100$$

Annual legume seed harvest

Seed reserves of annual legumes were sampled in the first summer of the experimental period from all plots other than those sown to pure lucerne swards. Soil from the surface 0.02 m was excavated from two quadrats per plot; each quadrat 1.5 m by 0.2 m with the long side perpendicular to the drill-rows. Samples from each plot were combined and transported back to the laboratory where seed and burr were sieved free of soil. Seed mass (kg/ha) was determined after threshing and cleaning.

Most probable number of rhizobia

Estimates of most probable number (MPN) of rhizobia were made for subterranean clover, strand medic and biserrula. Only treatments sown to the annual legumes in the alternate row

configuration (1 : 1) with lucerne were sampled at each site in spring of years 2 and 3. Six cores, 40 mm in diameter by 0.1 m deep, were taken from the annual legume row per plot and bulked, targeting areas where healthy plants were growing. Six additional cores from between the lucerne and annual legume rows, ~125 mm from where the annual legumes were sown in year 1, were bulked separately. Samples were transported fresh and refrigerated in the laboratory overnight. Clods were broken up by hand and samples homogenised before a 10-g subsample of soil was taken and mixed for 1 h in an end-over-end shaker with 100 mL deionised water. MPN was estimated by using a serial-dilution, plant-infection, nodulation-frequency test (Brockwell 1963) using the annual legume species sown to the particular treatment as the test plant.

Statistical analyses

Analysis of variance was conducted separately for three groups of treatments with five treatments in Group I (lucerne with subterranean clover), six treatments in Group II (phalaris with lucerne and/or subterranean clover), and four treatments in Group III (lucerne with various annual legume species). Lucerne only was used as a common treatment in all three group of treatments, and lucerne–subterranean clover (1 : 1) was included in both Groups I and III treatments for comparison.

All data are presented at the site level due to a large site \times treatment interaction. A complete randomised model was used to perform the analysis of variance in GENSTAT Release 18.1 (Payne 2012) for: seedling density at establishment; basal frequency in year 3; relative cumulative annual DM production; annual legume seed yield in year 2; annual legume regeneration in year 3; fixed N input by legume species; and MPN of rhizobia on annual legumes in Group III treatments. The seasonal herbage DM production for lucerne, phalaris and annual legumes over 3 years for each group of treatments were spline-fitted using a linear mixed model in ASReml (Gilmour *et al.* 2009). The fixed effects were site, pasture treatment, linear components of time trend, and their full combinations of interactions. The random effects were replicate, spline component of the time trend, and associated interactions. All terms were included in the model initially, but terms that failed to achieve statistical significance (at $P=0.05$) were excluded from the final model. The fixed effects were tested using the Wald statistical test and the random effects were tested using the residual maximum likelihood ratio test when necessary.

Results

Plant establishment and basal frequency

There were few significant differences in the initial establishment densities of sown annual legume species among sites. The initial density of subterranean clover was similar regardless of row configuration or companion species (28 plants/m² averaged across all treatments and sites) but was generally numerically higher than biserrula and lower than strand medic where the annual legumes were sown in alternate rows with lucerne (Table 3). The initial establishment of lucerne was also generally similar across row-configuration treatments when grown in binary mixtures with subterranean clover with an

Table 3. Initial seedling density (plant/m²) of sown species under different treatment groups at Mirrool (Mi), Lockhart (Lo), Wagga Wagga (WW), Eurongilly (Eu) and Burrumbuttock (Bu), NSW
n.s., Not significant ($P > 0.05$)

Treatment	lucerne				phalaris				annual legume						
	Mi	Lo	WW	Eu	Bu	Mi	Lo	WW	Eu	Bu	Mi	Lo	WW	Eu	Bu
Lucerne only	47.1	51.6	54.7	73.5	30.9	—	—	—	—	—	—	—	—	—	—
Lucerne-subterranean clover (1:1)	43.1	43.8	60.2	62.1	19.3	—	—	—	—	—	22.4	20.7	30.9	23.2	19.3
Lucerne-subterranean clover (1:2)	55.6	33.8	54.2	56.9	23.1	—	—	—	—	—	30.4	21.3	40.7	40.2	32.0
Lucerne-subterranean clover mix	41.6	30.4	44.7	62.7	18.2	—	—	—	—	—	25.6	30.7	30.7	31.9	27.6
Subterranean clover only	—	—	—	—	—	—	—	—	—	—	25.1	24.9	32.9	36.3	26.2
I.s.d. ($P = 0.05$)	n.s.	15.18	n.s.	n.s.	n.s.	—	—	—	—	—	n.s.	n.s.	n.s.	n.s.	n.s.
<i>Group I. Lucerne-subterranean clover</i>															
Phalaris-subterranean clover mix	—	—	—	—	—	48.4	56.0	56.4	112.5	43.6	24.7	28.4	39.1	33.6	22.9
Phalaris-subterranean clover (1:1)	—	—	—	—	—	37.1	50.9	49.6	112.7	31.3	24.2	30.0	28.0	31.6	31.3
Phalaris-lucerne (1:1)	25.8	17.8	23.3	32.4	12.4	14.7	28.7	23.3	64.8	22.2	21.6	26.0	36.7	32.1	17.1
Phalaris-lucerne (1:2)	24.7	19.6	24.9	28.8	9.1	24.7	22.7	23.8	38.5	18.0	24.4	30.2	38.7	29.8	16.7
Phalaris-lucerne mix	23.6	14.4	28.7	28.7	12.7	24.7	27.8	27.3	48.3	26.9	19.6	22.2	33.1	31.2	22.9
Lucerne only	47.1	51.6	54.7	73.5	30.9	—	—	—	—	—	—	—	—	—	—
I.s.d. ($P = 0.05$)	16.25	11.19	13.03	9.09	n.s.	14.31	14.88	6.96	19.13	13.88	4.52	n.s.	n.s.	n.s.	n.s.
<i>Group II. Phalaris-lucerne with subterranean clover</i>															
<i>Group III. Lucerne-annual legume</i>															
Lucerne-biserrula (1:1)	53.1	41.8	54.7	63.6	32.9	—	—	—	—	—	11.1	12.9	14.9	19.6	4.4
Lucerne-medic (1:1)	47.3	39.1	60.2	61.9	23.8	—	—	—	—	—	55.3	34.0	38.4	104.7	23.1
Lucerne-subterranean clover (1:1)	43.1	43.8	60.2	62.1	19.3	—	—	—	—	—	22.4	20.7	30.9	23.2	19.3
Lucerne only	47.1	51.6	54.7	73.5	30.9	—	—	—	—	—	—	—	—	—	—
I.s.d. ($P = 0.05$)	n.s.	n.s.	n.s.	n.s.	n.s.	—	—	—	—	—	4.73	11.12	16.54	17.46	9.77

average of 46 plants/m² observed across all sites. As expected, in the Group II treatments where lucerne seeding rates were halved when sown with phalaris (see Table 2), the initial density of lucerne at each site was approximately half that observed in the pure lucerne swards (Table 3). The initial phalaris density also was observed to be approximately double in the phalaris–subterranean clover treatments that received a full sowing rate of phalaris, compared with the phalaris–lucerne treatments where the sowing rate of phalaris was halved.

There were few differences in lucerne and phalaris basal frequency at the Burrumbuttock site during the experimental period. In the Group I treatments at all other sites, lucerne basal frequency was generally higher in the lucerne-only and lucerne–subterranean clover mix treatments where lucerne was sown in every drill row (Table 4). In the Group II treatments, lucerne basal frequency was generally highest in the lucerne-only treatment, reflective of the higher initial sowing rate than lucerne in treatments mixed with phalaris. Similarly, phalaris basal frequency was greatest where phalaris was sown at a higher seeding rate in the absence of lucerne. The exception to this was in the phalaris–subterranean clover (1 : 1) treatment, which often had a basal frequency similar to phalaris sown in mixtures with lucerne, despite the different initial seeding rate. There was no effect of annual legume species on lucerne basal frequency in

the Group III treatments, but lucerne basal frequency was consistently higher in the lucerne-only treatment than in the treatments in which lucerne was sown only in every second row (Table 4).

Herbage production

Highly significant site × pasture mix × time interactions existed in the herbage DM data (Table 5). Lucerne biomass in the Group I treatments was generally greatest where lucerne was sown in every row and least where it was sown in every second or third row (Fig. 2). Similarly, subterranean clover biomass was generally greatest where it was sown in every row and reduced as the number of sowing rows reduced. The exception was the lucerne–subterranean clover mix, where subterranean clover biomass was generally low compared with other Group I treatments (Fig. 2). In the Group II treatments, lucerne biomass was generally greater in the lucerne-only treatment, although at some sites at least, such as Wagga Wagga and Mirrool, there were indications in the final year of experimentation that total lucerne production was similar, or greater, in treatments that also included subterranean clover. Phalaris biomass was generally greater where it was sown at higher rates in the absence of lucerne. There were few consistent

Table 4. Basal frequency (%) of lucerne and phalaris in year 3 under different treatment groups at Mirrool, Lockhart, Wagga Wagga, Eurongilly and Burrumbuttock, NSW
n.s., Not significant ($P > 0.05$)

Treatment	Mirrool	Lockhart	Wagga Wagga	Eurongilly	Burrumbuttock	Mean
<i>Group I. Lucerne–subterranean clover</i>						
			Basal frequency of lucerne			
Lucerne only	28.7	34.2	22.1	41.1	19.8	28.0
Lucerne–subterranean clover (1 : 1)	23.3	30.4	15.5	21.6	16.7	20.5
Lucerne–subterranean clover (1 : 2)	17.6	22.7	11.8	17.8	14.0	15.9
Lucerne–subterranean clover mix	26.9	36.2	10.4	40.2	17.6	23.6
Subterranean clover only	–	–	–	–	–	–
I.s.d. ($P = 0.05$)	6.18	5.95	3.98	9.13	n.s.	3.35
<i>Group II. Phalaris–lucerne with subterranean clover</i>						
			Basal frequency of phalaris			
Phalaris–subterranean clover mix	41.8	27.8	38.0	38.9	23.1	34.6
Phalaris–subterranean clover (1 : 1)	28.4	20.7	28.2	36.7	21.1	27.2
Phalaris–lucerne (1 : 1)	34.7	14.0	20.7	24.9	10.4	20.9
Phalaris–lucerne (1 : 2)	15.1	5.6	19.1	10.7	16.0	14.2
Phalaris–lucerne mix	24.0	8.7	22.9	21.3	16.0	19.3
Lucerne only	–	–	–	–	–	–
I.s.d. ($P = 0.05$)	n.s.	8.81	6.42	n.s.	n.s.	4.39
			Basal frequency of lucerne			
Phalaris–subterranean clover mix	–	–	–	–	–	–
Phalaris–subterranean clover (1 : 1)	–	–	–	–	–	–
Phalaris–lucerne (1 : 1)	15.3	25.8	11.2	17.3	7.8	14.8
Phalaris–lucerne (1 : 2)	16.2	24.7	9.4	30.2	13.6	17.2
Phalaris–lucerne mix	20.4	23.3	9.3	20.0	9.1	15.3
Lucerne only	28.7	34.2	22.1	41.1	19.8	28.0
I.s.d. ($P = 0.05$)	3.72	n.s.	8.35	6.56	n.s.	4.57
<i>Group III. Lucerne–annual legume</i>						
			Basal frequency of lucerne			
Lucerne–biserrula (1 : 1)	16.4	8.3	11.9	13.9	9.0	11.7
Lucerne–medic (1 : 1)	16.2	6.2	13.8	12.8	7.0	11.0
Lucerne–subterranean clover (1 : 1)	17.0	7.3	13.8	12.3	8.3	11.7
Lucerne only	23.0	8.9	16.8	19.0	9.5	15.0
I.s.d. ($P = 0.05$)	4.01	7.28	n.s.	4.48	n.s.	4.75

Table 5. Wald test for herbage dry matter (DM) production under different treatment groups across five sites over 3 years from 2012 to 2014
d.f., Degrees of freedom; d.d.f., denominator d.f. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s., not significant ($P > 0.05$)

Strata/decomposition	Effect	Total DM		Lucerne DM		Phalaris DM		Annual legume DM		Weeds DM	
		d.f.	d.d.f.	d.f.	d.d.f.	d.f.	d.d.f.	d.f.	d.d.f.	d.f.	d.d.f.
<i>Group I. Lucerne-subterranean clover</i>											
Site	F	4	30.8	4	22	4	8.05***	4	31.9	4	17.4
Residual	R										
Pasture mix	F	4	572	3	6.3	4	57.28***	4	572.8	4	572.4
Site.pasture mix	F	16	566.2	12	455.8	12	8.80***	16	566.9	16	570.3
Residual	R										
Linear (time)	F	1	65.9	1	80.4	1	60.52***	1	185.5	1	406
Site.linear (time)	F	4	49.1	4	47.3	4	8.99***	4	89.5	4	169.1
Pasture mix.linear (time)	F	4	408.1	3	454.7	3	2.30n.s	4	397.2	4	558.3
Site.pasture mix.linear (time)	F	16	563.7	12	455	12	4.58***	16	564.4	16	571.3
Residual	R										
<i>Group II. Phalaris-lucerne with subterranean clover</i>											
Site	F	4	41.5	4	28.5	4	15.90***	4	219.7	4	10.4
Residual	R										
Pasture mix	F	5	703.4	3	7.3	4	66.75***	5	12.1	5	11
Site.pasture mix	F	20	707.6	12	453.2	16	5.18***	20	709	20	707.3
Residual	R										
Linear (time)	F	1	47.6	1	99.6	1	55.95***	1	234.6	1	599
Site.linear (time)	F	4	41.2	4	62.8	4	9.26***	4	103.6	4	282.1
Pasture mix.linear (time)	F	5	706.2	3	452.9	4	1.19n.s.	5	707	5	703.3
Site.pasture mix.linear (time)	F	20	705.2	12	451.8	16	5.51***	20	705.6	20	707.6
Residual	R										
<i>Group III. Lucerne-annual legume</i>											
Site	F	4	37.2	4	25.6	4	8.38***	4	18.6	4	13.4
Residual	R										
Pasture mix	F	3	442.6	3	6.6	3	58.96***	3	441.9	3	453.2
Site.pasture mix	F	12	436.9	12	431.5	12	5.80***	12	436.4	12	453.3
Residual	R										
Linear (time)	F	1	60.9	1	88.9	1	100.90***	1	272.5	1	387.4
Site.linear (time)	F	4	46.1	4	38.1	4	10.36***	4	115.5	4	180.9
Pasture mix.linear (time)	F	3	444.9	3	431.9	3	3.99***	3	445.5	3	455.3
Site.pasture mix.linear (time)	F	12	434.2	12	430.8	12	3.94***	12	435.4	12	455.6
Residual	R										

patterns across sites in the Group II treatments other than the fact that subterranean clover biomass was consistently lowest in the lucerne-only treatment (Fig. 3). In the Group III treatments, lucerne biomass was again greatest where lucerne was sown alone. Annual legume biomass was generally greatest at all sites in the lucerne–subterranean clover (1 : 1) treatment compared with the treatments sown to biserrula or strand medic (Fig. 4).

Relative cumulative annual herbage DM production at each site, which includes sown plus volunteer species, is presented in Table 6. There were few significant differences in total biomass during the experimental period at three of the five sites; Burrumbuttock, Eurongilly and Lockhart. Productivity of the pure lucerne and subterranean clover swards was inconsistent at the remaining two sites, often representing either the most or least productive treatment for a particular year compared with

other Group I or II treatments. At Mirrool and Wagga Wagga in particular, the lucerne–medic (1 : 1) treatment was often the least productive sward of the Group III treatments.

Sward composition and annual legume regeneration

The average botanical composition of the swards in the final year of the pasture phase is shown in Fig. 5. There were clearly large differences in pasture composition between sites, with lucerne comprising a much smaller percentage of the total sward at Burrumbuttock and Wagga Wagga than the remaining sites. The abundance of volunteer weed species was commonly highest in treatments initially sown to only one species: lucerne or subterranean clover. The abundance of weeds was also often numerically lower in treatments sown to phalaris,

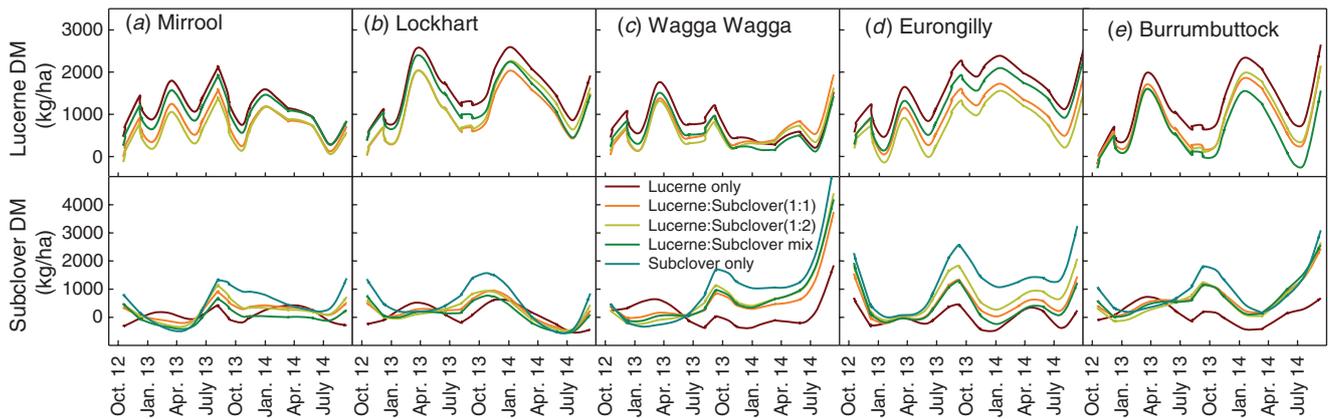


Fig. 2. Predicted lucerne and subterranean clover dry matter (DM) for Group I treatments over 3 years: (a) Mirrool, (b) Lockhart, (c) Wagga Wagga, (d) Eurongilly, and (e) Burrumbuttock, NSW. Drill-row ratios are in parentheses; note the different scales used on the y-axis.

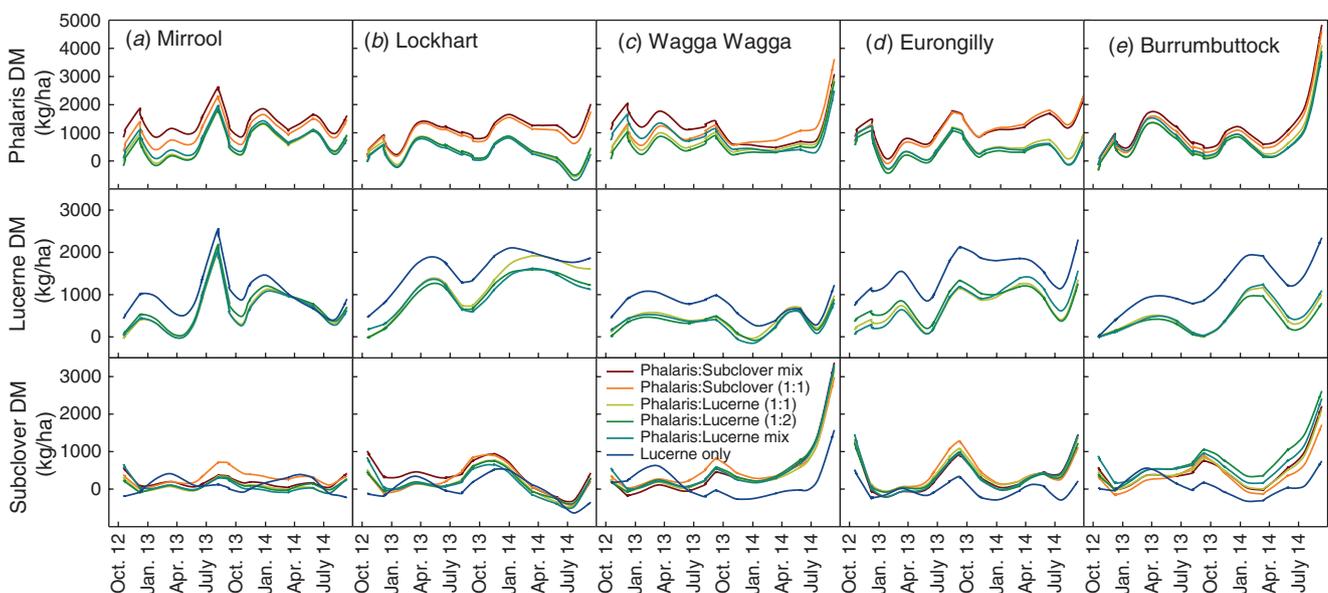


Fig. 3. Predicted phalaris, lucerne and subterranean clover dry matter (DM) for Group II treatments over 3 years: (a) Mirrool, (b) Lockhart, (c) Wagga Wagga, (d) Eurongilly, and (e) Burrumbuttock, NSW. Drill-row ratios are in parentheses; note the different scales used on the y-axis.

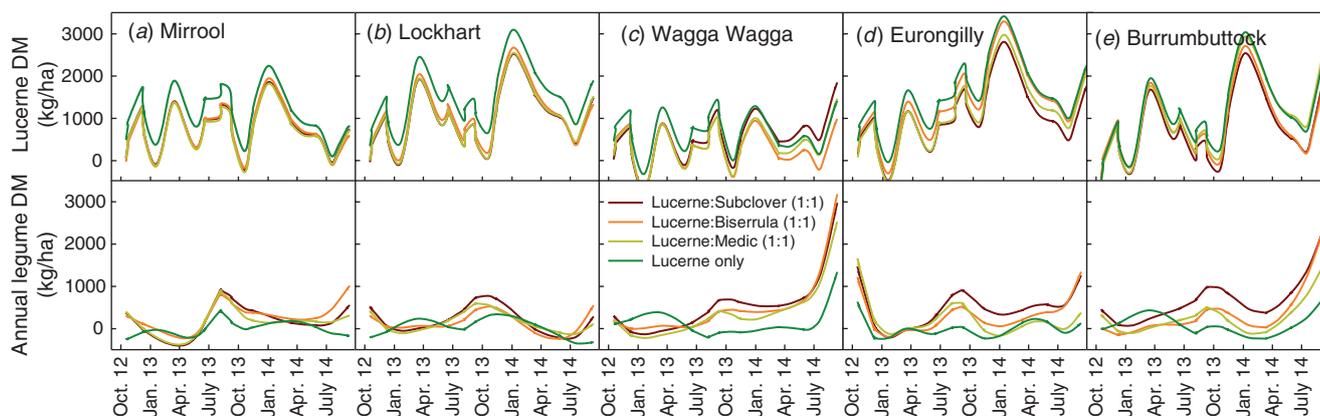


Fig. 4. Predicted lucerne and annual legume dry matter (DM) for Group III treatments over 3 years: (a) Mirrool, (b) Lockhart, (c) Wagga Wagga, (d) Eurongilly, and (e) Burrumbuttock, NSW. Drill-row ratios are in parentheses.

lucerne and subterranean clover than in binary mixtures of phalaris and subterranean clover where lucerne was excluded.

There was no significant difference in subterranean clover seed reserves during the first summer among the Group II treatments at any site. In the Group I treatments, subterranean clover seed reserves were generally lowest where that species was sown in mixed rows with lucerne (Table 7). Otherwise, at all sites except Burrumbuttock, the order of decreasing subterranean clover seed reserves mirrored the order of decreasing number of drill-rows sown to that species: Subterranean clover only > lucerne–subterranean clover (1:2) > lucerne–subterranean clover (1:1). Across all sites, biserrula had the highest seed reserves of any annual legume species in Group III. Seed reserves of the strand medic were numerically lower than subterranean clover at the Burrumbuttock and Wagga Wagga sites (Table 7).

Annual legume regeneration in autumn of year 3 (2014) is presented in Table 8. Across all sites in the Group I treatments, regeneration density in year 3 reflected patterns of subterranean clover seed reserves in year 1: subterranean clover only > lucerne–subterranean clover (1:2) > lucerne–subterranean clover (1:1) > lucerne–subterranean clover mix. Differences in subterranean clover regeneration between Group II treatments were significant ($P < 0.05$) only at the Wagga Wagga site with the greatest density observed where subterranean clover was sown in alternate rows with phalaris. Subterranean clover regeneration was consistently lower in all treatments sown to phalaris and lucerne than where subterranean clover was grown with phalaris alone. Patterns of annual legume regeneration in the Group III treatments were less consistent across sites, with higher densities of subterranean clover regeneration than of the other species at the Burrumbuttock and Wagga Wagga sites. By contrast, subterranean clover density was lower than strand medic and biserrula at the Lockhart site and there was no significant difference between species at the Mirrool site (Table 8).

Nitrogen fixation by legume species

The relative abundance of rhizobia compatible with the three annual legume species varied markedly with site. Rhizobia abundance was generally lowest at the drier site, Mirrool, and generally lowest for rhizobia compatible with biserrula compared

with either strand medic or subterranean clover. In almost all cases, MPN was greater for all legume species on the initial drill-row than between rows (Fig. 6).

Estimates of the proportion of legume herbage N derived from biological N_2 fixation (%Ndfa) for spring growth in 2013 and 2014 varied with legume species and site (detailed data not presented), but tended to be greater for subterranean clover (mean across all sites 74%) and biserrula (69%) than either stand medic (58%) or lucerne (54%). Inputs of fixed N were generally greater in treatments where lucerne was sown in combination with annual legume species than where lucerne was sown alone, or where phalaris was included in the pasture mixture (Table 9). Amounts of shoot N fixed were also calculated to be greater in the lucerne–biserrula (1:1) treatment than other Group III treatments (Table 9). The amounts of N_2 fixed varied from 15 to 34 kg N/t shoot DM (mean 26 kg N/t) across the five experimental sites. However, few consistent treatment effects were apparent apart from significantly higher levels of N fixed per t legume shoot DM accumulated in swards when phalaris was a component of the pasture, especially by subterranean clover (Table 9).

Discussion

This study examined relative pasture performance at five sites representative of the soil and climatic conditions of the mixed farming zone of south-eastern Australia, to understand whether: (i) alternate sowing configuration improved the balance between two companion perennial species, phalaris and lucerne; (ii) alternate sowing configuration improved the balance between those perennial species and subterranean clover; and (iii) a sulfonylurea-tolerant cultivar of strand medic or annual legume species with a high level of hard seed such as biserrula offered any production advantages over subterranean clover when grown in swards with lucerne.

Results showed that relative herbage yield from a mixed sward increased as the number of drill-rows of that sown species increased regardless of species. Planting mixed swards in monoculture rows, on the other hand, increased intra-specific competition and reduced interspecific competition because seed of a particular species was concentrated in one row

Table 6. Relative cumulative annual dry matter (DM) production (kg/ha) over 3 years under different treatment groups at Mirrool, Lockhart, Wagga Wagga, Euroungilly, and Burrumbuttock, NSW
n.s., not significant ($P > 0.05$)

Treatment	Mirrool			Lockhart			Wagga Wagga			Euroungilly			Burrumbuttock		
	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3
Lucerne only	1476	7386	2293	1229	7284	4769	1456	4100	5640	4653	5803	6520	595	8512	6664
Lucerne-subterranean clover (1:1)	863	7192	2827	1067	6472	4371	1039	4738	9049	4210	5411	6394	680	8117	8342
Lucerne-subterranean clover (1:2)	760	7258	2850	933	7253	5141	809	5014	9755	4416	5027	6999	407	8096	8186
Lucerne-subterranean clover mix	1397	7446	2494	2027	7057	4155	1499	4380	9287	4972	6212	7205	722	7394	8122
Subterranean clover only	868	4421	4607	1109	5906	4801	715	4726	10726	2964	5712	7825	888	7078	7885
I.s.d. ($P=0.05$)	n.s.	1622.6	1253.4	n.s.	782.1	n.s.	377.2	n.s.	846.5	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
<i>Group I. Lucerne-subterranean clover</i>															
Phalaris-subterranean clover mix	2444	9701	4889	2247	7032	4425	2993	5887	10232	5444	4606	8008	1607	7710	11602
Phalaris-subterranean clover (1:1)	1451	9164	4611	973	7298	4324	2002	6166	10619	4971	5345	7928	893	7405	9563
Phalaris-lucerne (1:1)	1339	9892	4515	1088	7912	5400	2074	5970	10056	6272	5203	7210	765	8507	10335
Phalaris-lucerne (1:2)	1311	10459	4904	748	7651	4294	1431	5514	10232	6222	5228	6200	570	8197	9834
Phalaris-lucerne mix	2161	10407	3814	1633	7157	3974	3679	5720	9334	5407	5379	6858	1728	8631	10776
Lucerne only	1476	7386	2293	1229	7284	4769	1456	4100	5640	4653	5803	6520	595	8512	6664
I.s.d. ($P=0.05$)	514.7	1850.3	769.6	613.5	n.s.	n.s.	742.1	907.5	760.8	n.s.	n.s.	n.s.	807.1	n.s.	n.s.
<i>Group II. Phalaris-lucerne with subterranean clover</i>															
Lucerne-biserrula (1:1)	884	7203	2825	991	6395	4081	1005	3606	9181	4656	5168	5523	354	8116	8211
Lucerne-medic (1:1)	896	6637	1937	901	6105	4472	878	3528	6524	4708	4433	4571	468	8678	7799
Lucerne-subterranean clover (1:1)	863	7192	2827	1067	6472	4371	1039	4738	9049	4210	5411	6394	680	8117	8342
Lucerne only	1476	7386	2293	1229	7284	4769	1456	4100	5640	4653	5803	6520	595	8512	6664
I.s.d. ($P=0.05$)	461.9	n.s.	577.1	n.s.	n.s.	n.s.	332.3	785.0	2079.4	n.s.	n.s.	1033.6	n.s.	n.s.	n.s.
<i>Group III. Lucerne-annual legume</i>															

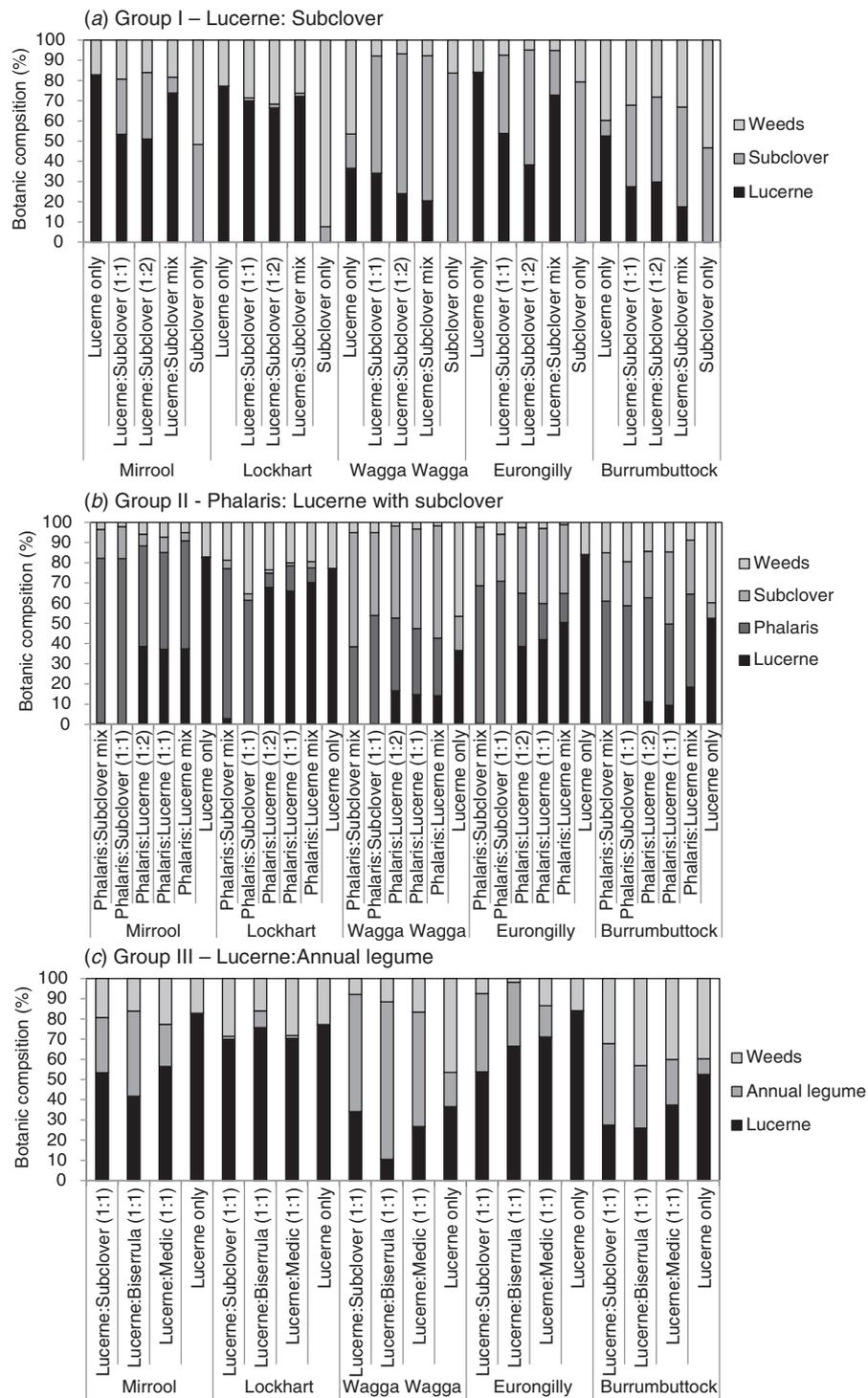


Fig. 5. Botanical composition (%) in year 3 (a) Group I, lucerne–subterranean clover, (b) Group II, phalaris–lucerne with subterranean clover, and (c) Group III, lucerne–annual legume at Mirrool, Lockhart, Wagga Wagga, Eurongilly and Burrumbuttock, NSW. Drill-row ratios are in parentheses.

(Harper 1977). In the present study, seeding rates within the drill-row were increased by up to 1.7 times compared with seeding densities where the species was sown in every drill row. Most studies of seedling competition do not consider

competition in terms of a linear row, but rather in multi-dimensional concepts of biological space relative to the position of a particular seedling. This is appropriate because seedlings intercept resources from all directions around them.

Table 7. Annual legume seed yield harvested in early summer in year 2 under different treatment groups at Mirrool, Lockhart, Wagga Wagga, Eurongilly, and Burrumbuttock, NSW
n.s, Not significant ($P > 0.05$)

Treatment	Mirrool	Lockhart	Wagga Wagga	Eurongilly	Burrumbuttock	Mean
<i>Group I. Lucerne–subterranean clover</i>						
Lucerne only	–	–	–	–	–	–
Lucerne–subterranean clover (1 : 1)	84.1	79.3	85.4	208.4	111.0	113.7
Lucerne–subterranean clover (1 : 2)	109.4	95.6	128.6	320.1	81.8	149.8
Lucerne–subterranean clover mix	70.0	45.9	71.7	134.6	104.2	85.3
Subterranean clover only	155.0	109.5	171.0	388.2	88.7	182.5
l.s.d. ($P = 0.05$)	n.s.	n.s.	n.s.	111.90	n.s.	51.76
<i>Group II. Phalaris–lucerne with subterranean clover</i>						
Phalaris–subterranean clover mix	62.5	81.1	59.9	88.3	20.1	62.4
Phalaris–subterranean clover (1 : 1)	99.6	36.9	65.8	207.3	49.5	91.8
Phalaris–lucerne (1 : 1)	61.6	74.8	48.8	114.4	70.3	74.0
Phalaris–lucerne (1 : 2)	76.0	79.3	59.5	155.5	83.1	90.7
Phalaris–lucerne mix	57.7	42.3	52.8	86.8	52.7	58.5
Lucerne only	–	–	–	–	–	–
l.s.d. ($P = 0.05$)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
<i>Group III. Lucerne–annual legume</i>						
Lucerne–biserrula (1 : 1)	273.6	147.7	123.6	491.9	96.5	226.7
Lucerne–medic (1 : 1)	94.0	115.7	36.4	275.8	56.9	115.8
Lucerne–subterranean clover (1 : 1)	84.1	79.3	85.4	208.4	111.0	113.7
Lucerne only	–	–	–	–	–	–
l.s.d. ($P = 0.05$)	137.40	n.s.	n.s.	188.90	n.s.	29.84

Table 8. Annual legume regeneration in year 3 under different treatment groups at Mirrool, Lockhart, Wagga Wagga, Eurongilly, and Burrumbuttock, NSW
n.s, Not significant ($P > 0.05$); n.a, data not available

Site	Mirrool	Lockhart	Wagga Wagga	Eurongilly	Burrumbuttock	Mean
<i>Group I. Lucerne–subterranean clover</i>						
Lucerne only	–	–	–	–	–	–
Lucerne–subterranean clover (1 : 1)	156	270	648	n.a.	707	445
Lucerne–subterranean clover (1 : 2)	211	382	628	n.a.	839	515
Lucerne–subterranean clover mix	93	203	556	n.a.	525	344
Subterranean clover only	342	506	1127	n.a.	757	683
l.s.d. ($P = 0.05$)	127.1	244.1	182.4	–	n.s.	99.2
<i>Group II. Phalaris–lucerne with subterranean clover</i>						
Phalaris–subterranean clover mix	82	402	420	n.a.	335	310
Phalaris–subterranean clover (1 : 1)	79	311	555	n.a.	343	322
Phalaris–lucerne (1 : 1)	48	362	346	n.a.	381	284
Phalaris–lucerne (1 : 2)	57	226	286	n.a.	455	256
Phalaris–lucerne mix	70	95	305	n.a.	639	277
Lucerne only	–	–	–	–	–	–
l.s.d. ($P = 0.05$)	n.s.	n.s.	117.7	–	n.s.	n.s.
<i>Group III. Lucerne–annual legume</i>						
Lucerne–biserrula (1 : 1)	251	348	462	n.a.	198	314
Lucerne–medic (1 : 1)	165	468	278	n.a.	139	262
Lucerne–subterranean clover (1 : 1)	156	270	648	n.a.	707	445
Lucerne only	–	–	–	–	–	–
l.s.d. ($P = 0.05$)	n.s.	60.3	137.8	–	193.7	29.8

The linear row is therefore simply a superficial structure imposed on agricultural plant communities for human convenience (Donald 1963). Nevertheless, the linear row governs the initial placement of agricultural seeds and seedlings, and is thus an important consideration in understanding population dynamics of pasture communities. It is likely that constraining the same

number of individuals to a smaller number of drill rows reduced the capacity of a species ‘population’ to access resources.

The exception in the present study was where a species was sown in every drill row along with seed of another, more dominant species, such as the lucerne–subterranean clover mix. Generally, lucerne biomass in this treatment was found to be

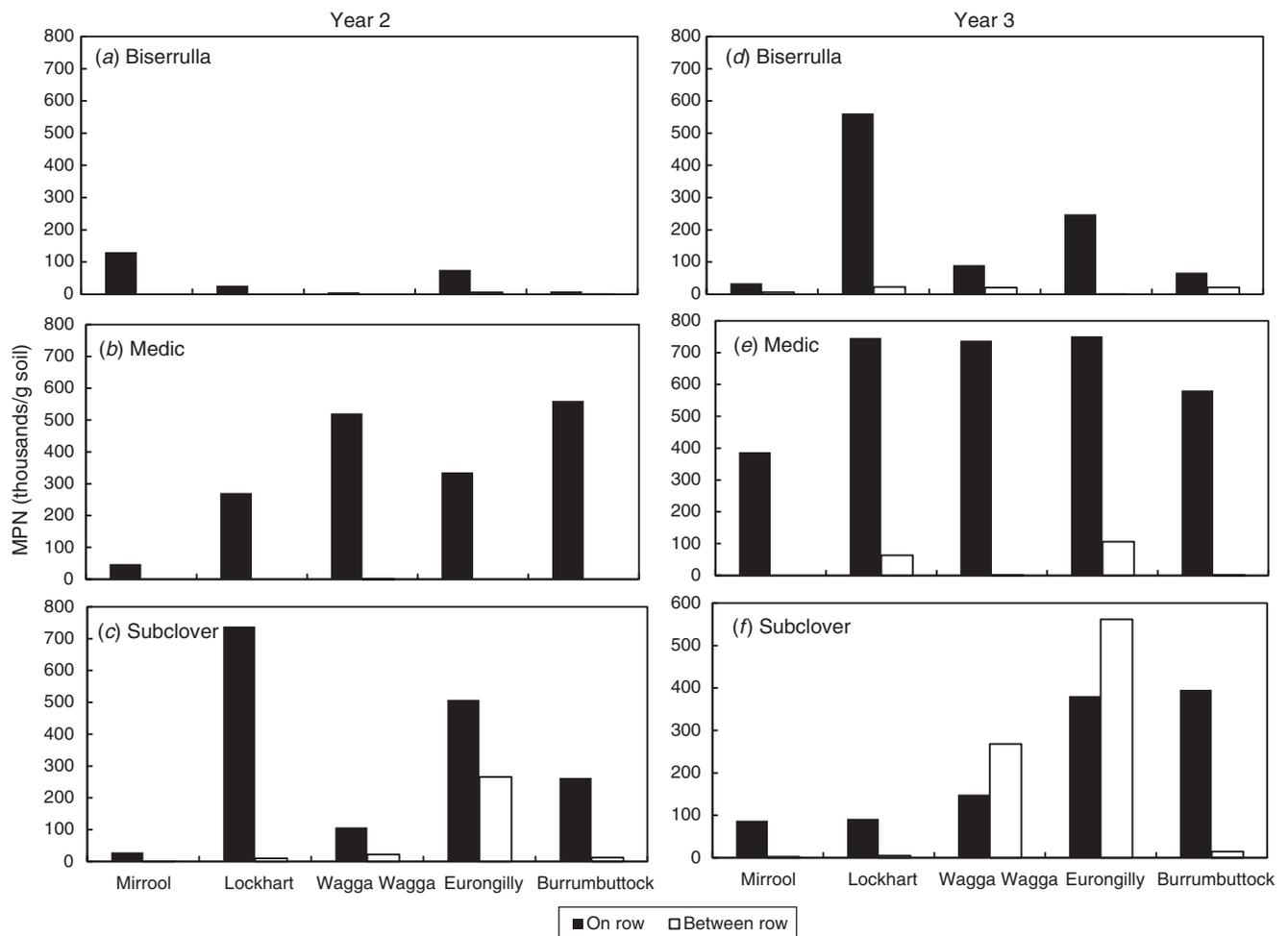


Fig. 6. Most probable number (MPN) of rhizobia on annual legumes under Group III treatments in October in years 2 and 3. Samples were taken on the row of annual legumes and between rows of lucerne and annual legumes.

second only to the lucerne-only treatment where lucerne was also sown in every drill row, but in the absence of interspecific competition from subterranean clover. However, subterranean clover yields in this treatment were among the lowest of any in its group despite subterranean clover also being planted in every drill row. This illustrates the balance between maximising species yield through exposure to resources and reduced intraspecific competition *v.* constraining the yields of more dominant species such as lucerne to enable the persistence of less dominant but equally desirable species such as subterranean clover. It is likely that the constrained subterranean clover biomass production in this treatment is a result of competition from the neighbouring lucerne population, which is consistent with previous experience in similar environments (Dear and Cocks 1997; Dear *et al.* 2000).

The question of species dominance is also situation-dependent, and there was evidence within our study of a different hierarchy at particular sites, even within the small range of pasture species tested. For example, lucerne appeared less dominant over subterranean clover at the Burrumbuttock and Wagga Wagga sites than at the other three locations. This was evident in the yield and botanical composition data where lucerne biomass comprised a lower proportion of the total

sward at these two sites (e.g. Fig. 5). The factors leading to this dominance shift are not known, although it is interesting that the annual *Medicago* species also yielded lowest in terms of total annual legume seed reserves at these sites. Low soil pH could be an explanation at the Burrumbuttock site, but it does not explain the poor performance of the medicos at the Wagga Wagga site (Table 1). The conclusion is that the effectiveness of alternate row sowing configurations in influencing interspecific competition will likely be site-dependent, and the outcomes may not always be predictable. This approach to pasture sowing should therefore be trialled on a small scale in a local environment before broadening to a commercial scale.

Effects of row configuration on dynamics between two perennial species

The total aboveground biomass responses of mixed phalaris–lucerne pasture swards to alternate row configurations were mixed. There was some evidence that alternate row configurations suppressed total production, particularly in year 1. In other instances, there was no significant difference in biomass yields regardless of sowing configurations. Yet, at

Table 9. Calculated inputs of fixed nitrogen by legume species, based on shoot N values averaged across two years in years 2 and 3, under different treatment groups at Mirrool, Lockhart, Wagga Wagga, Eurongilly, and Burrumbuttock, NSW

Values are presented on an area basis (kg N/ha) and expressed as a function of legume aboveground dry matter (DM) production (kg N/t DM). n.s., Not significant ($P > 0.05$)

Treatment	Mirrool kg/ha	kg/t	Lockhart kg/ha	kg/t	Wagga Wagga kg/ha	kg/t	Eurongilly kg/ha	kg/t	Burrumbuttock kg/ha	kg/t	Mean kg/ha	kg/t
<i>Group I. Lucerne-subterranean clover</i>												
Lucerne only	18	20	41	27	20	19	57	21	47	29	37	23
Lucerne-subterranean clover (1 : 1)	21	21	41	29	98	27	74	24	83	30	63	26
Lucerne-subterranean clover (1 : 2)	22	20	38	28	111	29	98	26	76	28	66	26
Lucerne-subterranean clover mix	21	22	30	26	97	27	83	22	77	27	59	25
Subterranean clover only	26	24	35	35	111	31	51	15	67	32	58	27
I.s.d. ($P = 0.05$)	n.s.	n.s.	7.4	4.6	26.0	3.9	27.3	4.9	17.0	n.s.	8.9	n.s.
<i>Group II. Phalaris-lucerne with subterranean clover</i>												
Phalaris-subterranean clover mix	7	24	18	34	67	33	33	27	46	32	35	30
Phalaris-subterranean clover (1 : 1)	15	31	15	34	55	31	28	26	38	34	30	32
Phalaris-lucerne (1 : 1)	19	23	35	26	61	27	89	28	48	27	46	26
Phalaris-lucerne (1 : 2)	19	22	33	26	73	30	72	27	71	29	52	27
Phalaris-lucerne mix	16	24	27	25	69	29	83	26	62	28	48	26
Lucerne only	18	20	41	27	20	19	57	21	47	29	37	23
I.s.d. ($P = 0.05$)	8.1	n.s.	7.7	4.1	20.6	2.6	30.7	3.6	n.s.	3.6	4.2	2.6
<i>Group III. Lucerne-annual legume</i>												
Lucerne-biserrula (1 : 1)	21	21	41	29	98	27	74	24	83	30	63	26
Lucerne-medic (1 : 1)	29	22	33	26	68	18	67	21	39	26	47	23
Lucerne-subterranean clover (1 : 1)	16	18	33	26	62	19	52	21	50	28	43	22
Lucerne only	18	20	41	27	20	19	57	21	47	29	37	23
I.s.d. ($P = 0.05$)	n.s.	n.s.	7.6	n.s.	18.5	2.9	n.s.	n.s.	22.6	2.6	7.7	n.s.

other times, alternate sowing configurations enhanced DM production compared with lucerne and phalaris sown together in the same drill-row. This range of results likely reflects the seasonality of production of the particular species in combination with the seasonal conditions experienced at the various sites. For example, year 1 biomass at most sites was strongly influenced by phalaris production, reflecting its more winter growth habit and earlier production than lucerne. Therefore, the treatments that constrained phalaris production through a reduced number of drill-rows sown to that species, such as phalaris–lucerne (1 : 1) and phalaris–lucerne (1 : 2) were at a yield disadvantage during the initial period compared with treatments where phalaris was sown in every drill-row.

The highest relative cumulative biomass production at most sites in most years was generally found in treatments within Group II, and usually in treatments sown to a three-way mixture of lucerne, phalaris and subterranean clover. This generally supports the proposition that greater species diversity sustains greater production over time (Jolliffe 1997; Picasso *et al.* 2011), especially where grasses are grown in association with legumes (Finn *et al.* 2013). The spatial separation of lucerne and phalaris appeared to offer production advantages at the Wagga Wagga, Mirrool and Lockhart sites in particular, because in year 3 cumulative production in the phalaris–lucerne mix treatment was significantly lower ($P < 0.05$) than the phalaris–lucerne (1 : 1) or (1 : 2) treatments. There was little evidence from the basal frequency data that changes in relative density of the two perennial species explained the differences in production observed in year 3, nor was the difference in herbage production attributable to a difference in the abundance of weeds. Our results are in general agreement with earlier research near Wagga Wagga, which showed that a mixture of species made it possible to maintain a substantial contribution to summer growth from lucerne as well as vigorous winter growth from phalaris and subterranean clover over a 3–4 year pasture phase, thus enhancing overall pasture production compared with less diverse pastures (Wolfe and Southwood 1980).

The levels of N_2 fixation observed in our study (Table 9) are comparable to other reports (Dear *et al.* 1999; Peoples *et al.* 2001, 2012; Unkovich *et al.* 2010). Calculated inputs of fixed N by legumes were generally lowest in treatments within Group II. This directly reflected the reduced total legume biomass, a consequence of the substantial phalaris component in most of those treatments. This was despite the amounts of N fixed per t legume shoot DM being higher for the subterranean clover or lucerne grown in the Group II treatments. This observation is consistent with previous studies in similar environments where phalaris was shown to be more competitive than legumes for soil mineral N (Dear *et al.* 1999). Only at one site in the present study, Lockhart, did the alternate row configuration of lucerne and phalaris lead to enhanced inputs of N compared with the two perennial species being grown together in mixed drill-rows.

Effects of row configuration on subterranean clover grown with lucerne or phalaris

There were clear advantages in growing subterranean clover in drill-rows spatially separated from lucerne. As previously described, subterranean clover yields in Group I were

generally depressed where it was planted in mixed rows with lucerne compared with plantings in 1 : 1 or 1 : 2 row configurations. Subterranean clover regeneration in year 3 was consistently lowest in the lucerne–subterranean clover mix swards than in other Group I treatments, generally reflecting seed reserves early in the pasture phase.

By contrast, advantages of spatially separating subterranean clover and phalaris were less clear. Herbage yields of subterranean clover were not consistently superior in the phalaris–subterranean clover (1 : 1) swards among the Group II treatments, and at only one site, Wagga Wagga, was the regeneration of subterranean clover in year 3 enhanced compared with where the two species were grown together in mixed drill-rows. This result was unexpected. Previous studies have indicated that phalaris is a vigorous competitor with subterranean clover, perhaps even more competitive than lucerne (Dear and Cocks 1997; Dear *et al.* 1998). Complementarity of the winter-growing subterranean clover might also have been expected to be greater with the summer-growing lucerne than the winter-growing phalaris, and the effects of separating the two supposedly more complementary species might have been expected to be less. We therefore expected the advantages of spatial separation to be as great, or greater, in subterranean clover when grown with phalaris, particularly because of the obvious advantages of spatial separation in reducing shading of the clover by the grass canopy (Dear *et al.* 1998). More research is required to improve understanding of the effects of spatially separating the lucerne and subterranean clover.

Biserrula and strand medic performance

Subterranean clover was generally the best performing of the annual legume species grown in alternate rows with lucerne. It often had the highest herbage yields, particularly at the higher rainfall sites, and averaged across sites had the highest seedling regeneration in year 3. There was some evidence that biserrula performance was improving with time; by year 3 it comprised a larger proportion of total sward herbage than subterranean clover at several sites (Fig. 5). Initial seed yields of biserrula certainly exceeded those of the other legumes at most sites.

The reason for the superior performance of subterranean clover over the other annual legumes is not clear. The present study rules out survival of rhizobia as a contributing factor because sufficient compatible rhizobia (at least ~100/g soil; Herridge 2008) were observed at most sites in each of the relevant swards (Fig. 6). Furthermore, there was no evidence of major constraints to N_2 fixation by biserrula or stand medic, in terms of either %Ndfa or kg N fixed/t shoot DM. Availability of adapted cultivars is a more likely factor in favour of subterranean clover. Many more cultivars of subterranean clover are available in Australia than of the other species (Lattimore and McCormick 2012), which enabled sowing of multiple cultivars of subterranean clover at each site, in contrast to only one cultivar of each of the other species. This likely enhanced the capacity of subterranean clover to adapt to seasonal variability experienced at each of the experimental sites. The improved relative performance of biserrula and strand medic at the lower rainfall sites supports the argument

that cultivar adaptation was an important factor in the present study, because both Casbah biserrula and Angel strand medic are early-maturing varieties suited to drier environments. In both cases, very few alternative cultivar choices were available when the present experiments were sown (Lattimore and McCormick 2012). Subsequent to the experiments being sown, barrel medic (*M. truncatula* Gaertn.) cultivars with demonstrated tolerance to residual herbicides were developed (Peck *et al.* 2015). Barrel medic, which has previously been shown to exhibit comparable yields to subterranean clover (Dear and Jenkins 1992), may be a better option for the Riverina region on soils at risk of herbicide residues than the herbicide-tolerant strand medic cultivar used in the present experiment.

Both Casbah biserrula and Angel strand medic also have very high levels of hard seed relative to the subterranean clover cultivars used. This may not have been an advantage in the present study where pastures were managed over a 3-year phase. Success with these species has been reported in other parts of Australia where conditions are hotter and drier and where hard seed breaks down more quickly, or where the pastures are managed in 'ley' rotations with crops rather than as continuous pasture phases (Nichols *et al.* 2007, 2012). Ley rotation systems are more difficult to achieve where perennial forage species such as phalaris and/or lucerne form a fundamental component of the forage system. The disadvantage of very high levels of hard seed is the substantial risk of depressed legume growth in year 2 because much of the hard seed has not broken down and thus the number of regenerating seedlings can be depressed. Of the species tested in the present study, subterranean clover remains the best annual legume to complement perennial forages in the target region of southern NSW.

Conclusion

Alternative spatial configurations had mixed effects on pasture performance and composition. There were obvious and consistent benefits to subterranean clover of spatially separating it from lucerne. Fewer benefits were observed from separating lucerne and phalaris, or even subterranean clover and phalaris. Generally, the relative productivity of a species was directly related to the number of drill-rows into which it was sown, presumably related to the ability of that species to capture resources. The present study showed a general increase in pasture productivity where lucerne and phalaris were grown together, but spatial separation of the perennial species had mixed effects on pasture performance, sometimes leading to enhanced productivity and at other times leading to reduced productivity. The variable results were attributable to differences in the seasonality of production of particular species experienced at the various sites. Consistent benefits of alternative spatial configurations on the dynamics of lucerne and phalaris grown together were not observed. Our study also demonstrated that the harder seeded annual legumes, biserrula and strand medic, were no more productive than subterranean clover when grown in alternate rows with lucerne. Excessive levels of hard seed for phased pasture rotations under the climatic conditions experienced during the experimental period, and the narrow selection of adapted cultivars of the alternative species

presently available for evaluation, are two likely reasons for the superior performance of subterranean clover observed at the five locations in southern NSW.

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Chapter 4

The legacy of pasture drill rows on soil chemical characteristics

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The legacy of pasture drill rows on soil chemical characteristics and subsequent wheat production

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Abstract

Aims To examine the legacy of pasture drill rows sown to various configurations of subterranean clover (*Trifolium subterraneum* L.), lucerne (*Medicago sativa* L.) and phalaris (*Phalaris aquatica* L.), on soil chemical characteristics and wheat (*Triticum aestivum* L.) production in the year following pasture removal.

Methods A field experiment comparing 10 pasture species combination treatments conducted on a Red Kandosol soil in a semi-arid environment in south-eastern Australia was monitored from the end of the pasture phase until harvest of the first wheat crop.

Quadrats were fixed to the soil surface to mark previous pasture drill rows. Soil within and between rows was sampled to 0.6 m at three times and analysed for mineral nitrogen (N), pH, total carbon (C) and available macronutrients, and related to wheat growth rate and yield.

Results Significantly higher total C, mineral N and plant-available potassium (K), and lower sulphur (S) concentrations, were still evident in the original pasture drill row after grain harvest compared to the inter-row area, four years after pastures were sown. Grain yield was 11% greater in wheat growing on the original pasture drill row compared to between rows. Pure lucerne swards had less mineralised N than mixed lucerne swards associated with reduced water in the soil profile.

Conclusions This study establishes the enduring legacy of pasture drill rows observed up to 14 months post-removal and highlights the opportunity to improve soil fertility through manipulation of pasture sowing configurations in a cropping field in south-eastern Australia.

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Keywords Pasture composition · Legumes · Perennial grass · Nitrogen · Wheat yield

Introduction

Spatially separating pasture species in different drill rows at sowing may be a convenient way of manipulating mixtures of multiple species. Some operators have used this approach to vary the composition of a pasture by increasing or decreasing the number of drill rows containing particular species (for example, Wolfe and Southwood

1980), whilst others have used spatial separation to manage interspecific competition in an attempt to maintain multiple desirable species in a sward over a longer term (Butler et al. 2011; Hayes et al. 2016; Hayes et al. 2017c). In either instance, it would seem that the practice of spatially separating sward components at sowing has practical and commercial applications, particularly given the low cost and relative ease with which farmers could adopt this strategy through relatively minor modifications to existing practices and machinery (Hayes et al. 2017a).

Previous studies that have evaluated the practice of spatially separating species in drill rows have limited their examination to measuring above-ground characteristics of the sown sward for 1–3 years following sowing. To fully appreciate the broader consequences of adopting this strategy, particularly in the context of phased pasture-crop rotations, the present study monitored changes in soil chemical characteristics and grain yield of a wheat (*Triticum aestivum* L.) crop sown in the year following removal of the pasture.

Different species might be expected to confer contrasting effects on soil properties and thus on subsequent crop growth. The pasture species examined in this study, lucerne (alfalfa; *Medicago sativa* L.), phalaris (Harding grass; *Phalaris aquatica* L.) and subterranean clover (*Trifolium subterraneum* L.), are from different functional groups and have contrasting growth habits and characteristics. For example, lucerne is a long-lived perennial legume with a deep tap root. Phalaris by contrast is a long-lived perennial grass with deep fibrous roots, while subterranean clover is a short-lived annual legume with a relatively shallow fibrous root system. At the very least, legumes might be expected to deposit greater quantities of nitrogen (N) into the soil (Dear et al. 1999; Peoples et al. 2012) which would alter soil N availability at the smaller spatial scale and perhaps lead to secondary changes in soil chemistry such as changed soil pH (Dear et al. 2009; Tang et al. 1998), while the perennial species, lucerne and phalaris, are likely to influence plant-available water deeper within the soil profile (Angus et al. 2001; Sandral et al. 2006).

If an enduring legacy of the pasture sowing row can be demonstrated and quantified, the practice of altering the spatial arrangement at sowing may be expanded in the future to incorporate principles of precision agriculture. For example, variable rate fertiliser technology has been developed to account for spatial variability in soil fertility and yield potential across commercial fields. This manuscript reports the findings of field experimentation

quantifying the effect of sowing configurations of different combinations of pasture species on soil characteristics and subsequent grain yield. The study tests three hypotheses: i) that prior pasture drill rows improve soil fertility compared to the inter-row area, ii) that contrasting pasture species have a different impact on soil fertility, and iii) that improved soil fertility in pasture drill rows lead to increased wheat production after the pasture is terminated.

Materials and methods

Site description

A field experiment was sown at Wagga Wagga, New South Wales, Australia (35°02'S, 147°22'E). The Wagga Wagga region has been used for crop and pasture production for over 100 years. Prior to the establishment of the experiment the field was sown to wheat as part of a typical cropping rotation for that region which included canola (*Brassica napus* L.), wheat and barley (*Hordeum vulgare* L.). The clay content of the soil was 29%, 49% and 54% at 0–0.1 m, 0.1–0.4 m and 0.4–0.6 m depths, respectively. Soil pH in CaCl₂ ranged from 4.48 in the 0.05–0.1 m depth to 6.37 at 0.4–0.6 m (Conyers et al. 2018). The A-horizon of the soil was estimated to extend to approximately 0.15 m depth and the B1-horizon, observed to be structureless, extended to 0.6 m depth (M. K. Conyers unpublished). Wagga Wagga is a semi-arid environment receiving on average ~540 mm rainfall annually with an equi-seasonal distribution (based on long-term monthly averages) throughout the year but with a slight bias towards the winter months (Dear and Ewing 2008).

Experimental design

The experiment was sown in May 2012. There were 10 pasture swards with various combinations of up to three pasture species (phalaris, lucerne, and/or subterranean clover) planted in contrasting spatial configurations (Table 1). Each plot contained 16 drill rows of pasture at a row spacing of 250 mm, established by two passes of an 8-tine cone seeder fitted with narrow points and press wheels. The pasture seeding rate of a particular species was kept the same on an area basis (kg/ha) regardless of row configuration. The alternate row (1:1) treatment was achieved by condensing from 16 to 8 drill rows into which seed of a particular species

was sown per plot, resulting in a doubling in seeding rate per drill row. In the double skip-row treatments, such as lucerne – subterranean clover (1:2) where one row of lucerne was sown to every two rows of subterranean clover, the subterranean clover was condensed from 16 to 10 drill rows per plot increasing seeding rate per drill row by 60%, and the lucerne was condensed from 16 to 6 drill rows increasing seeding rate per drill row by 167%. Further details and results from the initial pasture phase of the experiment are reported in Hayes et al. (2017c).

Pasture swards were sprayed 3 years later in September 2014 with glyphosate (360 g/L active ingredient) at 2 L/ha and kept fallow throughout the summer with follow-up applications of glyphosate to kill weeds, as required. Detailed sampling areas were marked at the time of pasture removal to enable crop performance to be related to the prior location of sown pasture rows. One steel mesh quadrat divided into 50 mm × 50 mm cells was laid over a representative area of each plot and fixed by steel pegs hammered into the soil. The size of the quadrat was 1.2 m × 1.2 m, except for in the 2:1 row configuration treatments (treatment numbers 103 & 109, Table 1) where the quadrat size increased to 1.2 m × 2.0 m to ensure that an adequate number of each pasture row type was included in the sample area. Each quadrat was mapped at the time of placement, when pasture residues were still visible and easily identifiable, to indicate the cell rows which lay directly over a pasture sowing row. Any area within the quadrat that was obviously unsuited to further sampling, such as holes from previous soil sampling, was marked to ensure that future sampling and analysis avoided such areas.

The sampling regime therefore explores two experimental factors; i) the initial pasture type, and ii) the spatial location relative to the original pasture drill row. The experimental design compares 10 ‘pasture swards’ (Table 1) and 14 ‘sampling locations’ (Table 2).

Soil sampling and analysis

Soil was sampled on three occasions; in spring 2014 (17 September) at the commencement of the fallow, in autumn 2015 (23 April) just prior to the sowing of a wheat crop, and in summer 2015 (15 December) after grain harvest. Two soil coring tubes, each 25 mm in diameter, were inserted at each respective sampling location, either on or between the original pasture drill rows. As the initial pasture experiment was sown on a

row spacing of 250 mm, samples taken from between the drill rows were approximately 125 mm from either original drill row. Cores were taken to a depth of 0.6 m, the base of the B1-horizon, and divided into 0–0.5, 0.5–0.1, 0.1–0.2, 0.2–0.3, 0.3–0.4 and 0.4–0.6 m depths. Soil from two cores were bulked for each depth. The surface two depths (to 0.1 m) capture the majority of the A-horizon at this site.

Soils were air-dried in a fan forced dehydrating oven set at 40 °C and passed through a 2 mm sieve. Coarse organic material and gravel too large to pass through the sieve were discarded from the sample. Soil pH was determined on a 1:5 soil:1 M KCl solution shaken for 1 h and measured at 20 °C. The same extractant was used for the determination of mineral N (ammonium + nitrate) concentration using a QuickChem® 8000 series flow injection analysis system. Total concentrations of soil carbon (C) and N were determined by dry combustion (Leco 1995) according to method 6B2b in Rayment and Lyons (2011). Colwell phosphorus and potassium (Colwell 1963) as well as plant-available sulphur (KCl-40; Blair et al. 1991) were determined at a commercial laboratory (Incitec Pivot, Werribee). Available soil water content was determined at the April and December samplings by weighing samples before and after drying at 40 °C, multiplied by the bulk density for each respective depth as determined by characterisation cores taken adjacent to the experiment. Plant-available water (PAW) in the 0–0.60 m was calculated as the difference between ASW in December and in April 2015.

Crop management and sampling

In the fixed quadrat area where no cultivation was possible due to the presence of the quadrat, small holes were punched in the soil surface to a depth of approximately 25 mm before seeds of wheat cv. Gregory were individually sown in each hole on 7 May. Seeds were sown either directly on the original pasture drill row or in the inter-row approximately 125 mm from the original drill rows, with one seed per respective cell of the fixed quadrat. A total of 216 cells were sown in each 1.2 × 1.2 m quadrat, giving a target density of 150 plants/m². A similar density was targeted in the larger 1.2 × 2.0 m quadrats in which 384 cells were sown. No fertiliser was incorporated with the seed in the fixed quadrat area at sowing but starter fertiliser was broadcast two days prior to sowing across all fixed quadrat areas at a rate equivalent to 15 kg/ha P and 5 kg/ha N.

Table 1 Description of pasture treatments at the Wagga Wagga site

ID	Treatment	Abbreviation	Cultivar and sowing rate	Description
101	Lucerne only	Luc_only	Lucerne (50% Aurora and 50% Genesis) at 3 kg/ha	Lucerne sown alone in every drill row
102	Lucerne:Subclover (1:1)	Luc_Sub1:1	Lucerne (50% Aurora and 50% Genesis) at 3 kg/ha, subterranean clover (33% Bindoon, 33% Riverina, 33% Coolamon) at 4 kg/ha	Lucerne and subterranean clover sown in alternate drill rows
103	Lucerne:Subclover (1:2)	Luc_Sub1:2	As for No. 102	Two rows of subterranean clover to every one row of lucerne
104	Lucerne:Subclover mix	Luc_submix	As for No. 102 & 103	Lucerne and subterranean clover sown together in every drill row
105	Subclover only	Sub_only	Subterranean clover (33% Bindoon, 33% Riverina, 33% Coolamon) at 4 kg/ha	Subterranean clover sown alone in every drill row
106	Phalaris:Subclover mix	Phal_submix	Sirolan phalaris at 3 kg/ha, subterranean clover (33% Bindoon, 33% Riverina, 33% Coolamon) at 4 kg/ha	Phalaris and subterranean clover sown together in every drill row
107	Phalaris:Subclover (1:1)	Phal_sub1:1	As for No. 106	Phalaris and subterranean clover in alternate drill rows
108	Phalaris:Lucerne (1:1) with subclover	Phal_luc1:1	Sirolan phalaris at 1.5 kg/ha, lucerne (50% Aurora and 50% Genesis) at 1.5 kg/ha, mixed with subterranean clover (33% Bindoon, 33% Riverina, 33% Coolamon) at 4 kg/ha	Phalaris and lucerne sown in alternate drill rows, subterranean clover sown in every row
109	Phalaris:Lucerne (1:2) with subclover	Phal_luc1:2	As for No. 108	Two rows of lucerne to every one row of phalaris; subterranean clover sown in every drill row
110	Phalaris:Lucerne mix with subclover	Phal_lucmix	As for No. 108	Phalaris, lucerne and subterranean clover sown together in every drill row

Adapted from Table 2 in Hayes et al. (2017a)

Initial seedling density was measured on 22 May 2015 by counting the number of cells in each fixed quadrat into which wheat was originally sown. The first three assessments of seedling herbage mass was undertaken by visually assessing (score of 1–10) seedling biomass within the fixed quadrat area on 29 May, 10 June and 26 June 2015. Visual scores were calibrated by destructively harvesting representative seedlings from the remaining plot outside of the fixed quadrat area. The fourth biomass assessment was a destructive harvest of individual seedlings across a transect in each fixed quadrat on 31 July, approximately 73 days after initial emergence when plants were at Zadock growth stage Z25–Z26 (Zadocks et al. 1974). These transects ran perpendicular to the original pasture drill row. Seedlings were cut just above ground level and seedlings from the transect that originated from the same sampling location (for example, all seedlings that were grown on a lucerne row) were grouped together in a bag and transported to the laboratory where they were washed, tiller number recorded and then dried in a fan-forced dehydrator set at 60 °C and weighed. Samples were

ground using a laboratory mill to pass through a 1 mm screen and herbage N concentration (%N) was determined by combustion (Leco 1995).

Peak spring biomass was determined at anthesis and sampled on 16 October, approximately 150 days after initial emergence, by harvesting another transect from each fixed quadrat, as described above. At least two rows of wheat remained intact between the transects sampled in July and October, to minimise effects on the subsequent harvest. Herbage dry matter (DM) and N concentration were determined using the methods described above. Nitrogen uptake (content) was calculated by multiplying the N concentration by the herbage DM.

The same methods were used for sampling and processing for grain yield. Samples were collected on 24 November after a 190 day period since emergence. In contrast to the previous destructive DM samplings which harvested only one transect, three pre-determined adjacent transects were harvested and composited according to the sampling location relative to the original pasture drill row. Care was taken to ensure that these transects were not adjacent to any transects that were previously

Table 2 Description of sampling location treatments compared in the analysis

ID	Treatment	Abbreviation	Description
201	Lucerne row	Luc row	A drill row initially sown purely to lucerne
202	Lucerne/subterranean clover mixed row	Luc/sub row	A drill row initially sown to a mix of lucerne and subterranean clover
203	Phalaris row	Phal row	A drill row initially sown purely to phalaris
204	Phalaris/lucerne/subterranean clover row	Phal/luc/sub row	A drill row initially sown to a mix of phalaris, lucerne and subterranean clover
205	Phalaris/subterranean clover row	Phal/sub row	A drill row initially sown to a mix of phalaris and subterranean clover
206	Subterranean clover row	Sub row	A drill row initially sown purely to subterranean clover
207	Between lucerne rows	B/n Luc	Area between drill rows initially sown purely to lucerne
208	Between subterranean clover rows	B/n Sub	Area between drill rows initially sown purely to subterranean clover
209	Between lucerne/subterranean clover rows	B/n Luc/sub	Area between drill rows initially sown to a mix of lucerne and subterranean clover
210	Between lucerne and subterranean clover rows	B/n Luc_Sub	Area between a drill row sown purely

Table 2 (continued)

ID	Treatment	Abbreviation	Description
			to lucerne and another sown purely to subterranean clover
211	Between phalaris/subterranean clover rows	B/n Phal/sub	Area between drill rows initially sown to a mix of phalaris and subterranean clover
212	Between phalaris and subterranean clover rows	B/n Phal_Sub	Area between a drill row sown purely to phalaris and another sown purely to subterranean clover
213	Between lucerne/subterranean clover and phalaris/subterranean clover rows	B/n Luc/-sub_Phal/sub	Area between a drill row sown to a mix of lucerne and subterranean clover and another sown to a mix of phalaris and subterranean clover
214	Between phalaris/lucerne/-subterranean clover rows	B/n Phal/Luc/Sub	Area between drill rows initially sown to a mix of phalaris, lucerne and subterranean clover

harvested destructively. The number of tillers was counted, and grain was removed using a stationary thresher. Straw and grain was retained for analysis of N concentration. Harvest index was calculated by dividing grain yield by total above-ground biomass and relative kernel size was determined by weighing a subsample of 100 seeds per sample. Yields were expressed as g/m² and calculated assuming a plant density of 137 plants/m², based on the initial assessment of seedling emergence.

Apparent net in-crop N mineralisation (ANM) was calculated as the sum of residual soil mineral N measured in December 2015 plus wheat N accumulated in the above- (measured) and below-ground (calculated) biomass, minus the initial soil mineral N determined in September 2014 according to the following equation:

$$\text{ANM} = [\text{Soil mineral N (Dec 15)} + \text{accumulated crop N}] - \text{initial soil mineral N (Sep 14)} \quad (1)$$

In the calculation of ANM for this particular experiment it was assumed that N losses from the system were negligible based on direct measurements of gaseous and leaching losses of N undertaken at a nearby field site in 2013 and 2014 (Li et al. 2016), and long-term simulation model predictions (Smith et al. 2019).

Statistical analysis

Principal component analysis was conducted initially to identify likely associations between pasture type or sampling location. The first examined associations of treatments based on crop yield components at the end of the experimental period, including grain yield, grain protein concentration, harvest index, kernel size and above-ground biomass. The second examined associations based on soil data at the three sampling times, including pH, mineral N, total N, total C, and plant-available P, K and S. Multidimensional scaling analysis using the sets of variables listed above and assuming Euclidean distances was conducted in R 3.3.3 (R Core Team). Data was averaged by pasture type or sampling location and rescaled by subtracting the means and dividing by the standard deviations to minimise the influence of data with larger magnitudes. A hierarchical cluster analysis that assumed average linking was conducted on the same data.

Further analysis was conducted using either pasture type (plot level) or sampling location (drill row level) as a fixed factor. The number of replicates varied with treatment due to the sampling regime, as this varied for each treatment in order to properly represent the full treatment. Briefly, all mixture and pure sward treatments (numbers 101, 104, 105, 106, 110; Table 1) were sampled at two locations, both on and between the drill row; all 1:1 treatments (numbers 102, 107, 108, Table 1) were sampled at three locations, on each row type and between the

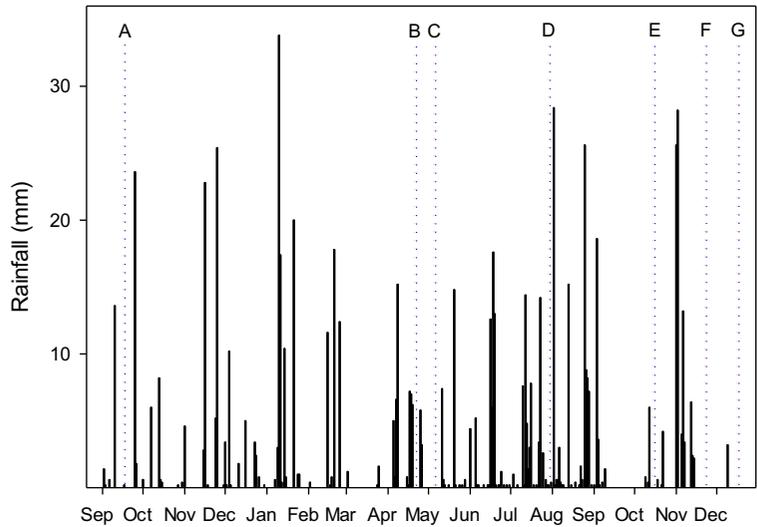
rows; and the 1:2 treatments (numbers 103, 109; Table 1) were sampled at four locations, on both row types as well as between the drill rows sown to the same species and between the drill rows sown to different species. With three true replicates at the plot level multiplied by the number of sampling locations, the number of replicate samples in the analysis varied between 6, 9 and 12. Similarly, the number of replicates at the drill row level varied according to the number of treatments that included a particular sampling location. For example, only three replicates existed for row types that were unique to just one treatment, such as treatments 203, 204, 207, 211, 212 and 214 (Table 2). Four row types (numbers 208, 209, 210 and 213) existed in two treatments providing 6 replicates of data, and three row types (201, 202, and 205) existed in three treatments providing 9 replicates of data. Only one sampling location, the subterranean clover drill row, existed in 4 treatments giving a total of 12 replicates of data. Much of the data presented averaged across all ‘on row’ treatments ($n = 45$) and all ‘between row’ treatments ($n = 36$) in an analysis of variance using Genstat 18th edition (VSN International, Hemel Hempstead U. K.) with sampling location, sampling date, and soil depth as the fixed effects and replicate and pasture type as the random effects. Least significant differences were calculated at the 5% level.

Results

Rainfall

Total rainfall in 2015 was 602 mm (Fig. 1), 11% above the average total received annually (541 mm) at the Wagga Wagga Agricultural Institute, 3 km from the field site, based on records from 1898 to 2015. A total of 323 mm of rainfall was recorded in the 7-month fallow period between pasture removal and sowing wheat (from September to April), constituting close-to average total rainfall for that period. Total growing season rainfall (from May to November) for the wheat crop in 2015 was 373 mm; however, very little spring rainfall was received with only 37 mm cumulative rainfall occurring in September and October compared to the long-term average cumulative rainfall for both months of 100 mm at that site.

Fig. 1 Daily rainfall (mm) at the Wagga Wagga site during the experimental period, September 2014–December 2015. Key dates are denoted by red dotted lines as A) Initial soil sampling and herbicide application to pasture B) Soil sampling prior to crop establishment C) Planting of wheat crop D) Initial sampling of crop N content E) Sampling of crop biomass at anthesis F) Harvest of crop at maturity, and G) Final soil sampling



Treatment groupings

A hierarchical cluster analysis with seven soil variables including soil mineral N, total N, total C, pH and plant-available P, K and S in the 0–0.6 m of the profile at three sampling times separated the Phalaris:Subclover (1:1) from the remaining pasture treatments (Fig. 2a). There were further groupings of the nine remaining pasture treatments but these did not appear to be based on any obvious differences in species composition. A second cluster analysis (Fig. 2b) based upon sampling location showed two groupings of treatments which clearly distinguished

the ‘on-row’ locations from the ‘between-row’ locations.

The hierarchical cluster analysis for the harvest variables including grain yield, grain protein, harvest index, grain size and final above-ground biomass, revealed that the subterranean clover only and the Lucerne:Subclover (1:1) pasture types clustered separately from all other treatments. The remaining pasture treatments that included lucerne and subterranean clover or lucerne, phalaris and subterranean clover generally clustered together, separate from the treatments that included phalaris and subterranean clover. The pure lucerne

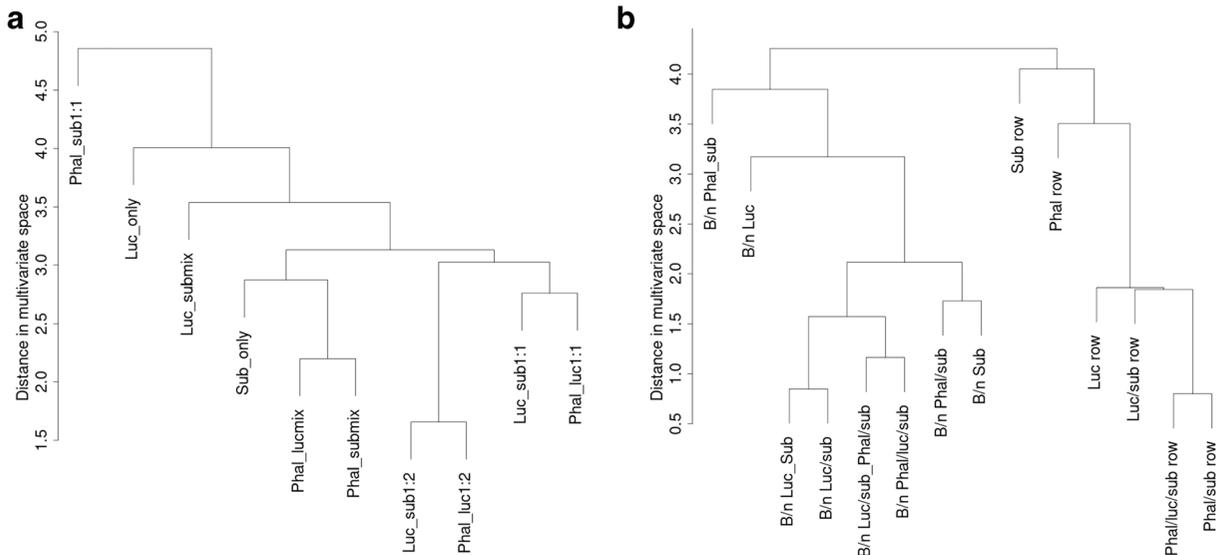


Fig. 2 Relationship between a) pasture treatments or b) sampling location following multivariate analysis that included soil pH, mineral N, total N, total C, available P, available K and available S sampled to 0.60 m at three sampling dates

treatment clustered most closely with the phalaris/subterranean clover treatments (Fig. 3a). The hierarchical cluster of the sampling location revealed that the locations between subterranean clover rows and between lucerne rows and subterranean clover rows clustered separately to the remaining treatments, within which there appeared to be few groupings consistent with species or sampling location (Fig. 3b).

Soil characteristics

There were no significant effects of previous pasture type treatment on soil mineral N to 0.60 m depth at any sampling date ($P > 0.05$). However, mineral N concentration changes over the three sampling dates differed significantly ($P < 0.001$) according to the sampling location relative to the original pasture sowing row. Relatively low quantities of soil mineral N were available to 0.60 m depth in September 2014 and December 2015. The highest soil mineral N was observed in April 2015 following 7 months of fallow (Fig. 4 a, d, g). Regardless of the species sown, the lowest mineral N values in April were observed on the original pasture drill row (range 82–131 kg/ha cf. the between-row values 143–226 kg/ha). Mineral N below 0.1 m was often greatest under lucerne drill rows compared to phalaris and subterranean clover drill rows, although

differences were not always significant at $P = 0.05$ (data not shown).

Total soil C concentration (% LECO) at the three sampling times varied with depth ($P < 0.001$) and sampling location ($P < 0.05$), but not due to original pasture treatment. Approximately 65% of soil total C was observed in the surface 0.10 m of soil. The soil total C values, at the surface (0–0.10 m), were significantly ($P < 0.05$) higher under the original pasture drill row compared to the inter-row. Differences attributable to the pasture drill row were still observed at the end of the first year of crop, although appeared diminished compared to previous samplings (Fig. 4 b, e, h). Total soil C concentration was significantly ($P < 0.05$) greater in the surface depth at the April 2015 sampling (2.60%) compared to the September 2014 and December 2015 samplings (both 2.24%).

Soil pH_{KCl} at the three sampling times varied with depth ($P < 0.001$) and sampling location ($P = 0.004$). Soil was most acutely acidic in the 0.05–0.10 m zone (pH_{KCl} 4.05, averaged across all treatments and dates) and generally only mildly acidic below 0.40 m (pH_{KCl} 5.66). Soil pH was largely consistent across the three sampling times, except in the surface 0.05 m where, averaged across all treatments, values were significantly ($P < 0.05$) lower at the April sampling (average 4.89) compared with those in September (5.26) and December (5.14). Differences due to sampling location were only

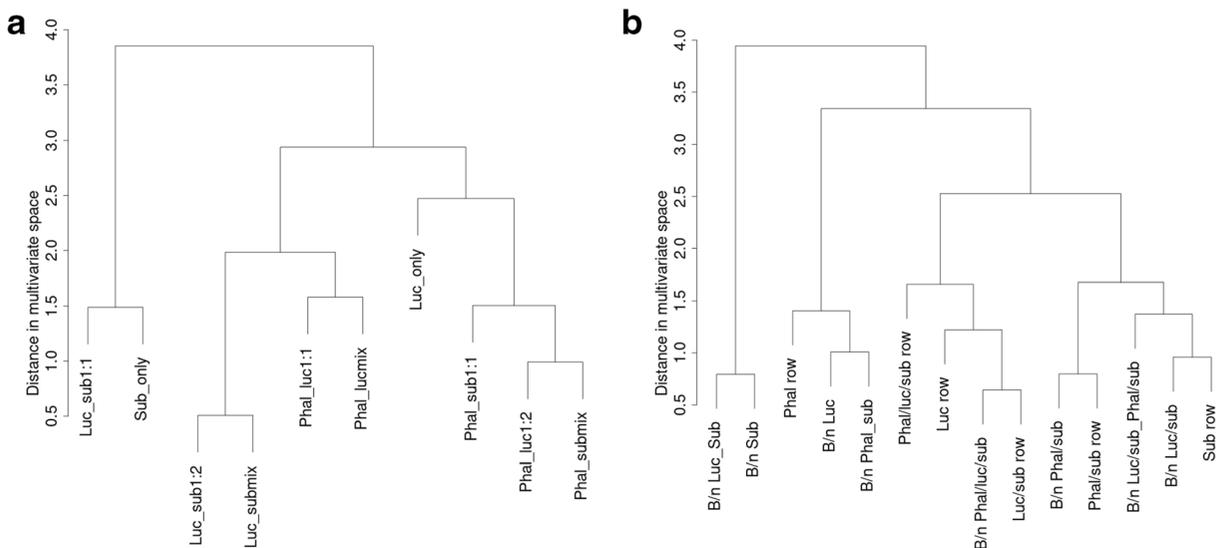


Fig. 3 Relationship between a) pasture treatments or b) sampling location relative to the original pasture drill row following multivariate analysis that included grain yield, grain protein concentration, harvest index, aboveground biomass and kernel size

significant in the surface 0.1 m depth at the first sampling time. Soil pH was 0.3 units greater under drill rows compared to the inter-row and was greater ($P < 0.001$) under drill rows containing a perennial plant than under subterranean clover drill rows (Fig. 5). In the 0.05–0.10 m zone soil pH was significantly greater under the pure lucerne drill row compared to other drill rows but this effect diminished with time and was not observed at the final sampling. At the plot level, there was no effect of initial pasture treatment on soil pH at any sampling date.

Plant-available soil P was significantly lower in soil under pasture drill rows at the end of the pasture phase in the surface 0.05 m only but these differences were not observed at the subsequent sampling times (Fig. 6 a, d, g). Values of available P were numerically lower under the subterranean clover-only drill row (28.7 mg/kg) in

the 0–0.05 m layer at the September sampling compared to all other drill rows (average 37.1 mg/kg) although differences were not significant ($P > 0.05$) due to large error terms. Available soil P levels below 0.2 m depth were generally at levels below accurate detection (< 5 mg/kg) at the September sampling and therefore were not analysed at the April or December samplings.

Plant-available soil K was up to 50% greater ($P < 0.05$) under the drill row compared to the inter-row in the surface 0.2 m of soil at the end of the pasture phase (Fig. 6 B). Differences to 0.1 m depth were still statistically significant at the end of the first year of crop, although were not as large as those observed at the first sampling (Fig. 6 e, h). Available K was significantly lower ($P < 0.05$) under the subterranean clover-only row (372 and 301 mg/kg, at the 0–0.05 m and 0.05–0.1 m depths, respectively) compared to

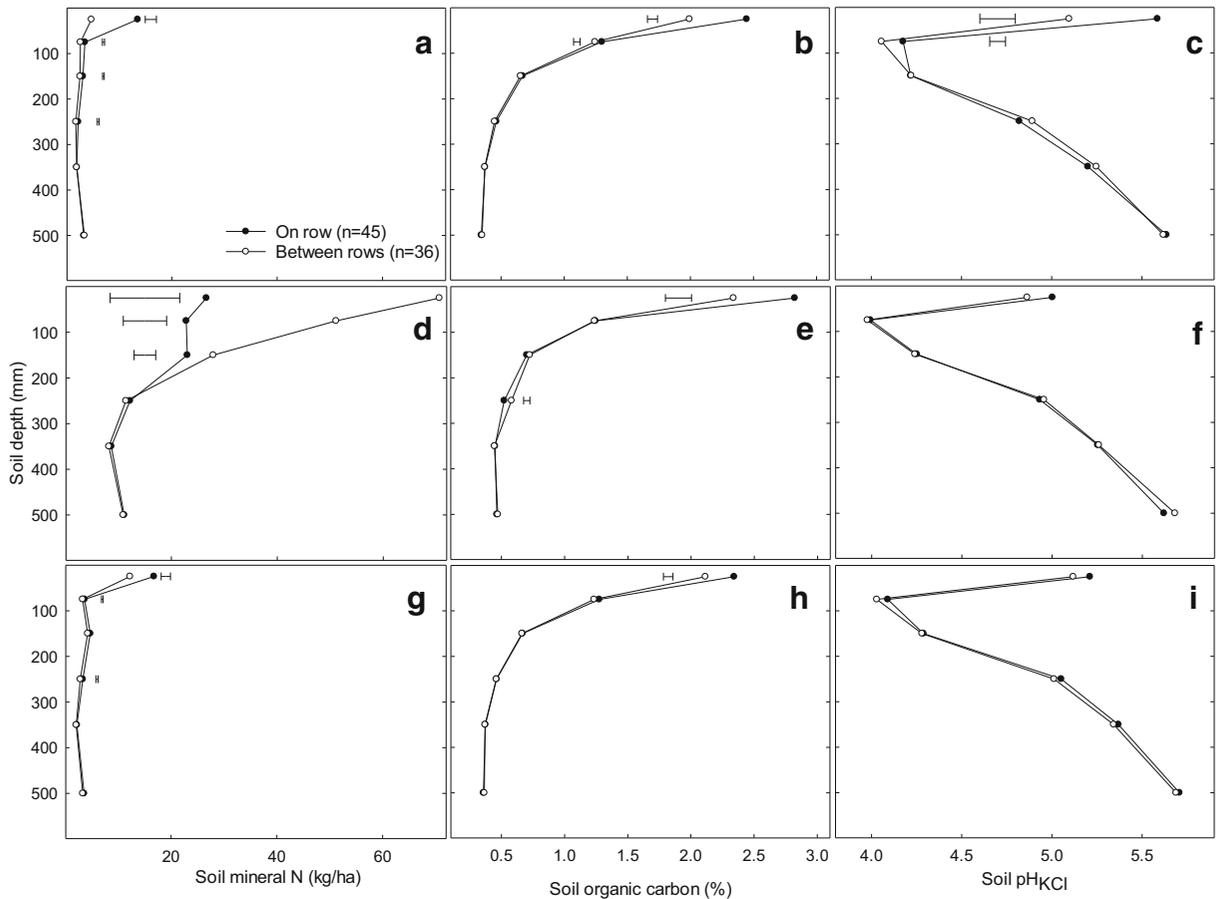


Fig. 4 Soil mineral N (a, d, g), organic carbon (b, e, h) and pH (c, f, i) at six depths in the surface 0.60 m profile at sampling locations comparing soil under pasture drill rows with the inter-row area in

September 2014 (a, b, c), April 2015 (d, e, f), and December 2015 (g, h, i). Differences are not significantly different at $P = 0.05$ unless marked with an l.s.d. error bar

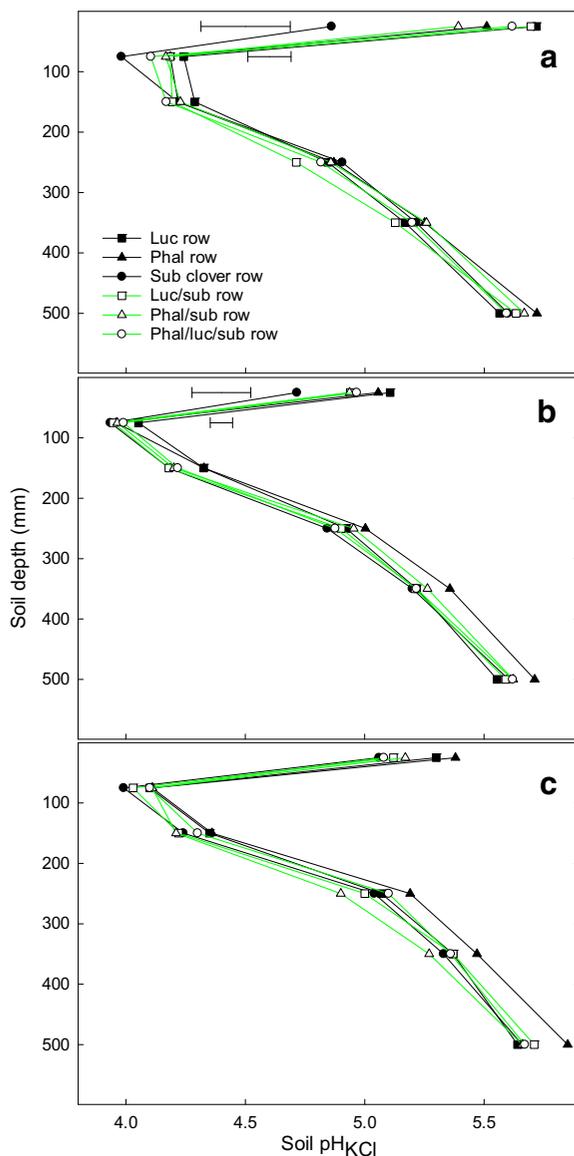


Fig. 5 Comparison of soil pH_{KCl} at six depths in the surface 0.60 m profile at sampling locations under drill rows sown to lucerne (luc), phalaris (phal), subterranean clover (sub) or mixtures thereof sampled in September 2014 (a), April 2015 (b), and December 2015 (c). Differences are not significantly different at $P = 0.05$ unless marked with an l.s.d. error bar

drill rows sown to perennial plants (706 and 395 mg/kg, respectively) and was similar to the average of inter-row sampling location in the surface 2 depths at the September sampling (data not shown). These differences were still observed at the end of the fallow in the surface 0.05 m only, although differences between the subterranean clover drill row

(467 mg/kg) were not significantly different to values under the phalaris row (500 mg/kg) at this depth (average of other drill rows, 596 mg/kg). At the December sampling K values in the 0–0.05 m layer under the subterranean clover drill row remained numerically lower than all other drill rows but differences were not significant at this time ($P > 0.05$).

Plant-available soil S was significantly ($P < 0.05$) greater at 0–0.05 m under the pasture drill row compared to the inter-row locations at the end of the pasture phase but in the deeper depths to 0.3 m, were significantly greater in the inter-row compared to under the drill rows (Fig. 6 c). Available S under the subterranean clover-only drill row (7.4 mg/kg) at 0–0.05 m at this sampling time was significantly lower than values under all other drill rows (average 11.6 mg/kg). Available S was consistently greater at all depths to 0.3 m at the end of the fallow in the inter-row compared to drill row locations (Fig. 6 f) with no significant differences between pasture drill rows at any depth at this time (data not shown). At the December sampling, differences in plant-available soil S between the drill row and inter-row areas had diminished in the surface 0.1 m but were still evident below this depth, including at the 0.3–0.4 m zone where differences had not previously been observed (Fig. 6 i).

Gravimetric water content was determined on soils sampled in April and December 2015 at the 6 sampling depths to 0.6 m. Significant ($P < 0.05$) treatment differences in gravimetric water content existed at each depth due to pasture treatment and to sampling location relative to the original pasture drill row, except at the 0.1–0.2 m depth where no treatment differences were observed (data not shown). No treatment differences were observed in water content in soil samples taken at the end of the crop growing season in December. Using the December values as a proxy for plant lower limit, plant available water (PAW) leading into the crop growing season was calculated for each pasture treatment. There was 20–25 mm water available to the wheat crop in the 0–0.6 m layer of soil in April under all pasture treatments except under the pure lucerne treatment which was substantially drier with only 11 mm of PAW at 0–0.6 m (Table 3). Soil, both under and between pure lucerne rows, was significantly drier than that at any other sampling location (Table 3).

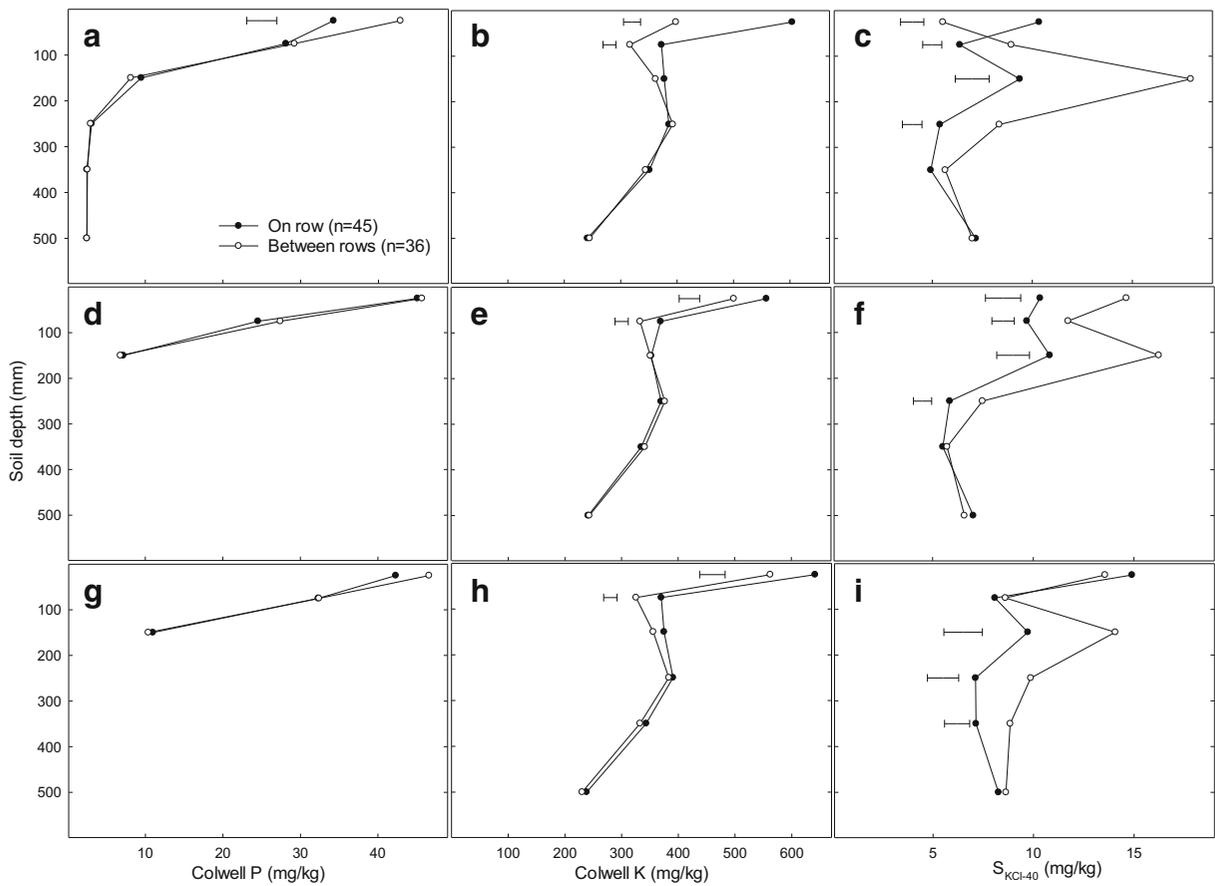


Fig. 6 Soil available phosphorus (Colwell; a, d, g), potassium (Colwell; b, e, h) and sulphur (S_{KCl-40} ; c, f, i) measured in the surface 0.60 m in September 2014 (a, b, c), April 2015 (d, e, f) and December 2015 (g, h, i) under pasture drill rows compared with the between-row area. Differences are not significant at $P = 0.05$

unless marked with an l.s.d. error bar. Values of Colwell P below 0.20 m depth at the September sampling (a) were generally below limits of accurate detection and were not analysed in the subsequent samplings (d, g)

Wheat establishment, herbage mass and shoot N concentration

There was no treatment effect ($P > 0.05$) on the number of wheat seedlings that emerged initially (data not shown). Averaged across all pasture type and sampling location treatments, approximately 91.5% of all wheat seeds sown in the fixed quadrat areas emerged as seedlings, equating to an average plant density of 137 plants/m².

Seedlings of wheat growing in original pasture drill rows were generally larger than those growing in the inter-row area throughout the growing season (Fig. 7). Early seedling DM (to six weeks post emergence) at the plot level was significantly ($P < 0.05$) higher in the pure lucerne plots and at the sampling location level, on the original drill rows sown only to lucerne on two of the

three sampling dates. There was no significant difference in tillering at the July sampling with an average 6–7 tillers per plant.

There was no effect ($P > 0.05$) of sampling location on the N concentration of wheat tissue by the fourth harvest in July, but wheat growing in the pure lucerne plots had significantly ($P < 0.01$) lower N concentrations than plants growing in plots previously sown to other pasture types. Wheat herbage was observed to have the greatest N concentrations (average 5.17%) in the Lucerne:Subclover(1:2) and Lucerne:Subclover(1:1) treatments, but was lowest in the pure lucerne (4.54%) and pure subterranean clover (4.76%) treatments. The wheat N concentration in the Phalaris:Subclover(1:1) treatment (4.67%) was similar to that of pure lucerne (l.s.d._{0.05} = 0.357). However, there was no effect ($P > 0.05$) on the calculated N content of wheat

Table 3 The effect of pasture treatment and sampling location relative to the original pasture drill row on plant available water (PAW; mm) in the surface 0.6 m prior to sowing the crop, above

ground N content (kg N/ha) of wheat sampled in July and November (maturity) 2015, and on calculated total N uptake of the wheat crop (kg N/ha) at maturity

Treatment	PAW (mm)	Above-ground N content (kg N/ha)		Total N uptake (kg/ha)
		July 2015	Nov 2015	Nov 2015
<i>Pasture treatment</i>				
Lucerne only	11.4	64.3	111.8	175.6
Lucerne:Subclover (1:1)	20.6	75.4	137.9	216.6
Lucerne:Subclover (1:2)	23.2	77.5	139.8	219.5
Lucerne:Subclover mix	20.7	70.2	144.7	227.2
Subclover only	19.5	67.2	124.5	195.4
Phalaris:Subclover mix	25.5	64.8	108.8	170.8
Phalaris:Subclover (1:1)	22.9	63.6	100.7	158.1
Phalaris:Lucerne (1:1) with subclover	24.8	67.4	122.6	192.5
Phalaris:Lucerne (1:2) with subclover	24.6	72.4	114.2	179.2
Phalaris:Lucerne mix with subclover	19.8	66.4	140.5	220.6
<i>l.s.d.</i> _{0.05}	5.03	–	28.55	22.47
<i>Sampling location</i>				
Lucerne row	15.6	81.6	144.9	227.5
Lucerne/sub clover mixed row	22.8	75.2	137.7	216.1
Phalaris row	27.4	56.6	102.1	160.4
Phalaris/Lucerne/sub clover row	20.7	59.6	149.0	234.0
Phalaris/sub clover row	24.4	64.1	110.4	173.3
Sub clover row	22.8	70.8	127.2	199.8
b/n Lucerne rows	12.3	58.5	96.6	151.7
b/n Sub clover rows	22.4	65.8	126.0	197.8
b/n Lucerne/sub clover mixed rows	22.1	71.0	121.4	190.5
b/n Lucerne and sub clover rows	20.5	75.4	128.7	202.0
b/n Phalaris/sub clover mixed rows	25.8	64.7	108.6	170.5
b/n Phalaris and sub clover rows	22.5	64.2	96.1	150.9
b/n Lucerne/SC and Phalaris/SC rows	26.6	68.6	118.0	185.3
b/n Phalaris/Lucerne/SC rows	19.2	73.2	131.9	207.2
<i>l.s.d.</i> _{0.05}	6.69	–	21.02	33.00

Abbreviation: b/n, between, SC, subterranean (or sub) clover

herbage at this time, either due to the initial pasture type or sampling location relative to the original pasture drill row (Table 3).

There was also no significant ($P > 0.05$) effect of pasture type or sampling location on wheat biomass at anthesis (average 9.8 g/plant). The N concentration of shoots at this time was significantly ($P < 0.01$) greater in the pure subterranean clover (1.11%) and lucerne/subterranean clover treatments (average 1.03%) but was

lowest in the pure lucerne treatment (0.80%). Significant differences in N concentration were also observed due to sampling location where values were greatest in shoots from plants growing between original subterranean clover rows (1.17%), and least in plants growing between pure lucerne rows (0.78%). Calculated N content of wheat herbage was not significantly ($P > 0.05$) affected by the original pasture type or sampling location at anthesis (data not shown).

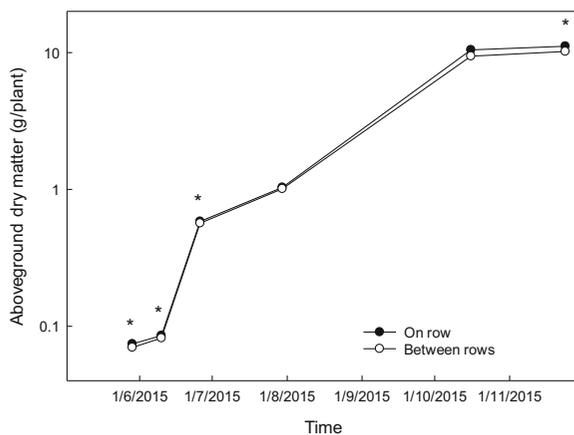


Fig. 7 Aboveground biomass of wheat (emergence date ca. 20 May 2015) from 29 May–24 November comparing plants grown on old pasture drill rows with those in the inter-row area. Differences are significant ($P < 0.05$) on dates marked with an asterisk (*)

Grain harvest

There was no significant difference in wheat DM at harvest, either at the pasture type or sampling location levels ($P = 0.43$ and 0.17 , respectively). After grouping treatments together for analysis, wheat plants growing on the original pasture drill row were 9% larger (11.1 g/plant) compared to plants growing in the inter-row area (10.2 g/plant; $P < 0.05$). There was also no significant difference in the number of heads per plant ($P > 0.05$). Grain yield increased by 11% on the drill row compared to the inter-row area ($P = 0.02$). Assuming a plant density of 137 plants/m², this equates to a grain yield increase from of 4.6 t/ha to 5.1 t/ha.

There were significant pasture type and sampling location effects on kernel size which was observed to be negatively correlated with grain N concentration (Fig. 8). Wheat grown following phalaris treatments tended to have a larger kernel weight but a lower grain N concentration relative to wheat following pure legume swards, except for wheat following a pure lucerne sward. Similarly, grain taken from locations that were on or between previous phalaris drill rows tended to be larger and lower in N. Significant ($P < 0.05$) treatment differences in harvest index closely reflected relative differences in grain size (see y-axis in Fig. 8). The highest harvest index (36.2%) was observed in wheat grown after a pure lucerne sward (treatment 101, Table 1), and the lowest (30.8%) in plots formerly sown purely to subterranean clover (treatment 105). Similarly,

the sampling location that was observed to have the highest harvest index was between pure phalaris and subterranean clover rows (treatment 212, Table 2; 36.2%) and was lowest (30.6%) between subterranean clover drill rows (#208).

Total N uptake was determined as the sum of grain N content, straw N content plus a calculated value for below-ground N, which was assumed to be equivalent to 36% of total N content of wheat i.e. close to half the N measured in above-ground parts (Wichern et al. 2008). Total N uptake was generally greater in wheat following mixed pasture swards that included lucerne and lower following pastures sown to pure lucerne or to phalaris/subterranean clover mixtures (Table 3). Total N uptake was also generally greater in wheat that was grown directly on previous pasture rows containing lucerne and/or subterranean clover. The highest total N uptake value was observed in wheat growing directly on a drill row previously sown purely to lucerne (227.5 kg N/ha) whilst one of the lowest values was from between pure lucerne rows (151.7 kg/ha).

Apparent net N mineralisation

Differences in ANM during wheat growth due to initial pasture treatment were significant ($P = 0.007$). In general, apparent in-crop net N mineralisation was greater in treatments sown with a mixture of lucerne and subterranean clover and lowest in the phalaris/subterranean clover and pure lucerne treatments (Table 4). Apparent net mineralisation differences due to sampling location were not significant ($P > 0.05$), however, they tended to be greater on original drill rows (210 kg/ha) compared to between drill rows 196 kg/ha; $P = 0.07$).

Discussion

The legacy of pasture drill rows, established in 2012, remained beyond the end of the pasture phase and at least until the end of the first wheat growing season nearly 4 years after pastures were sown. At the end of the pasture phase, soil mineral N, pH, total C and plant-available K were higher and plant-available P was lower under the original drill row compared to the inter-row area, with differences being greatest in the A-horizon. Plant-available S changed with depth, being greatest under the drill row at the soil surface, but less abundant deeper in the

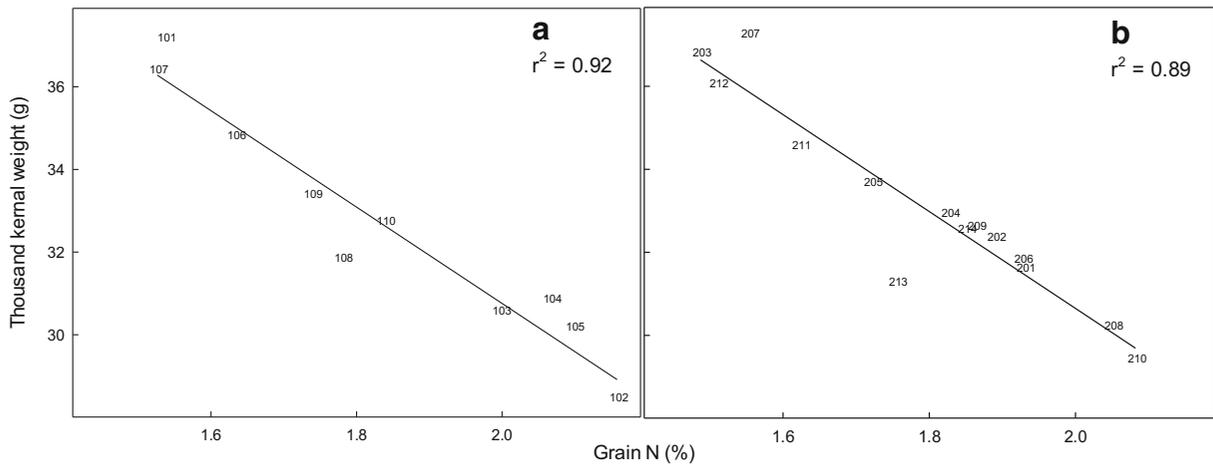


Fig. 8 The relationship between grain size, expressed as the mass of 1000 kernels (g), and grain nitrogen concentration as it relates to (A) original pasture treatment (see Table 1), or (B) sampling location (see Table 2)

soil profile under the drill row compared to the inter-row. All of these responses were indirect effects of increased pasture growth in the drill row, given that no fertilisers were applied in the drill row during the experimental period. Pasture density remained largely concentrated around the original drill row until the end of the pasture phase, including the self-regenerating annual subterranean clover (Hayes et al. 2020 and unpublished data), which explains the greater accumulation of organic matter on

rows compared to the inter-row area. The response of plant-available P being more depleted under the drill row likely reflects the fact that it is more limiting throughout the soil profile at this site compared to other minerals such as plant-available K but also may reflect different uptake mechanisms by plants of the different macro-nutrients which allows easier and more direct access to S and K by the root hairs than to P. Indeed, P uptake is governed by diffusion which can easily be interrupted by periodically

Table 4 Contribution of crop components and residual soil mineral N to calculated apparent net N mineralisation (ANM) under different pasture treatments in the 15 months from the end of the pasture phase until the end of the first wheat crop

Treatment	Crop values (kg N/ha)				Residual soil mineral N (kg N/ha)		ANM (kg N/ha)
	Grain	Straw/chaff	Belowground	Total N uptake	Sep 2014	Dec 2015	
Lucerne only	85.4	26.4	63.7	175.6	20.9	26.7	181.4
Lucerne:Subclover (1:1)	95.1	42.8	78.6	216.6	22.6	35.5	229.5
Lucerne:Subclover (1:2)	99.8	40.1	79.7	219.5	23.3	28.9	225.2
Lucerne:Subclover mix	102.2	42.5	82.5	227.2	24.7	34.9	237.4
Subclover only	85.8	38.7	71.0	195.4	21.7	33.7	207.4
Phalaris:Subclover mix	79.4	29.4	62.0	170.8	19.5	29.2	180.5
Phalaris:Subclover (1:1)	75.9	24.9	57.4	158.1	19.0	30.4	169.6
Phalaris:Lucerne (1:1) with subclover	92.7	29.9	69.9	192.5	29.0	31.5	195.0
Phalaris:Lucerne (1:2) with subclover	82.3	31.9	65.0	179.2	23.2	31.9	187.9
Phalaris:Lucerne mix with subclover	102.0	38.5	80.1	220.6	23.0	32.3	229.8
<i>l.s.d.</i> _{0.05}	18.11	8.32	14.09	22.47	—	—	39.68

dry soil conditions (Alam 1999), and P is known to be less prone than other nutrients to lateral and vertical movement in soils.

Soil chemical data indicated substantial levels of mineralisation during the summer fallow between September 2014 and April 2015. This is observed most readily in the mineral N data which increased in April compared to the September sampling regardless of sampling location but was substantially greater in the inter-row area. The 3-fold difference in mineral N at the April sampling in the inter-row compared to the drill row locations is likely a combination of increased incidence of finer root particles and fallen leaves in the inter-row space that were more easily decomposed, as well as increased organic matter in the original drill row that may continue to immobilise mineral N after the termination of the pasture. It is interesting that this substantial increase in the abundance of mineral N near the soil surface at the beginning of the growing season in the inter-row area did not translate into increased early crop growth. Indeed, seedling growth was greater on the drill row area despite the lower mineral N concentration, indicating that N was not the most limiting factor at this time. Both soil pH and Colwell K were greater under the drill row at the commencement of the crop growing season and may have contributed to biomass response observed in the wheat seedlings.

Annual and perennial species

The contrast in soil chemical data between drill rows sown only to subterranean clover compared to those sown to phalaris or lucerne was not expected and is worth exploring. Plant-available soil P, K and S were all lower at the soil surface under the subterranean clover drill row at the end of the pasture phase, although differences in soil P were not statistically significant ($P > 0.05$). In contrast to lucerne which is a perennial legume and phalaris, a perennial grass, subterranean clover is a self-regenerating annual legume. Reduced available nutrients in the soil surface may reflect the shallower rooting nature of subterranean clover compared to the deep-rooting perennial species, and the increased requirement for that species to satisfy its nutrient requirement from the surface layers of the soil. Greater volumes of roots at depth in phalaris and lucerne would afford these perennial species greater opportunity to supplement their nutrient requirement with some uptake of nutrients from depth. This would occur more

readily with S and K than with P which was shown to have very low availability below 0.2 m depth at this site.

The pH of surface soil under the subterranean clover drill row was more similar to pH in the inter-row than it was to other drill rows, and significantly lower than soil under either the lucerne or phalaris drill rows. This finding is in general agreement with Dear et al. (2009) where they measured soil pH at the end of a pasture phase and compared values close to the base of a perennial plant to those in gaps between perennial plants. Soil pH in their study was lower in the gap than at the base of a perennial plant, and lower in the subterranean clover plots. Dear et al. (2009) attributed these differences to apparent leaching of mineral N based on samplings taken in the final year of pasture in June, August and October and reported increased mineral N available in the soil surface of annual pasture plots compared to plots sown to perennial-based swards. Nitrification is commonly a major driver of soil acidification in these soil types (Helyar 1976) and although it was not measured directly in the present study, the relative similarity in mineral N values at the soil surface under the different drill rows does not suggest mineral N alone to be the cause of the pH differences observed. It is noted that the 15-fold increase in mineral N values in the inter-row from the start to the end of the summer fallow period did not correspond to changes in soil pH in this soil despite significant rainfall events during the fallow. Soil pH under the drill rows declined by 0.5 pH units between the first and second samplings compared to an average reduction of 0.2 pH units during the same period in the inter-row. We do not dispute the results of Dear et al. (2009), but evidence from our study would suggest that the mechanisms driving this relatively large change over a short period are not due to mineral N alone.

The addition of plant residue is another possible driver of pH change but again this may not fully explain the differences in pH between the drill rows sown to subterranean clover and those sown to other species. The addition of plant residues has an alkalising effect on surface soils (Paul et al. 2001) and the liming effects of plant shoots is much greater than that of plant roots (McLay et al. 1997). If plant residue returns explained the difference in the present study, residues from lucerne and phalaris would need to have been far greater than from subterranean clover. Although residue returns were not measured directly, seasonal biomass production at this site suggested that subterranean clover was the more productive of the three species (Hayes et al.

2017c). Ash alkalinity is also unlikely to explain the differences in pH change as lucerne is generally known to have higher ash alkalinity than subterranean clover (Slattery et al. 1991).

We suggest that a more likely explanation for the differences observed in surface soil pH between the subterranean clover and other drill rows is associated with the perennial growth habit of lucerne and phalaris. Specifically, it is perhaps not that subterranean clover reduced soil pH but rather, the presence of perennial plants increased soil pH during the pasture phase. Neither our study nor that of Dear et al. (2009) recorded an initial data point showing the pH profile at the start of the pasture phase and so we can only speculate. However, the sequence of pH profiles recorded in our study from the end of the pasture phase through to the end of the first crop year reveals that the difference between the different drill rows diminishes after the first sampling, attributable to an apparent increase in pH under the subterranean clover drill row of 0.2 pH units between the first and last samplings, and a reduction of 0.1–0.4 pH units under the drill rows planted to perennials over the same timeframe. It is impossible to isolate the effects of temporal and spatial variability that would inevitably impact such comparisons over time (Conyers and Davey 1990; Conyers et al. 1997) so those values need to be treated with caution. In the case of the Dear et al. (2009) study, sampling regime leading to greater alkaline plant material in the samples taken close to the perennial base would likely have impacted the results. The same may apply to our samples although sampling on the drill row was no guarantee that we were sampling in close proximity to a perennial plant and any differences that may have existed in plant residues were not detected in measures of total C. Further research is warranted to determine whether this apparent increase in soil pH in close proximity to perennial plants is a genuine shift in the chemistry of the soil or whether it is merely an artefact of a sampling regime that captures more plant residues, although our study shows that shifts in pH due to the presence of particular species are relatively short-lived beyond the end of the pasture phase.

Crop responses

The 11% increase in wheat yield from the pasture drill row compared to the inter-row area highlights the enduring legacy of the previous drill row into the cropping

phase and invites inquiry as to the likely mechanisms driving this response. The result is not likely to be due to increased availability of mineral N, P or S as all of those macro-nutrients were generally more available in the crop growing season in the inter-row area than the drill row. Plant-available K was more abundant on the drill row and cannot be ruled out as a contributing factor, although it seems difficult to credit this response to K which was at soil concentrations in excess of those previously shown to foster high biomass yields (Gourley et al. 2019). Soil pH is unlikely to explain the response for several reasons despite the inter-row area being more acidic than the drill row: i) differences in pH were only evident at the soil surface and only at the initial sampling 7 months prior to the crop being sown, ii) although acidic this soil type generally has low levels of manganese and aluminium likely to be toxic to plants (Hayes et al. 2012, 2017b), and iii) the soil pH of the drill rows formerly sown to subterranean clover was more acidic than drill rows sown to perennial species, yet there was no corresponding difference in grain yield between the different drill rows. The yield response is more likely to be attributed to the consistently higher levels of organic C observed in the surface soil throughout the experimental period which fostered a more abundant and diverse microbiome at the initial sampling (Hayes et al. 2020), and no doubt fostered a soil environment more conducive to plant growth.

Multivariate analysis of grain harvest components showed reasonably consistent groupings at a plot level based on species sown, most likely explained by differences in N supply. For example, apparent net N mineralisation during the first 14 months following pasture removal was lowest in the treatments sown purely to lucerne or to a mixture of phalaris and subterranean clover. This might be anticipated for lucerne due to slower net N mineralisation as a result of the coarser structure of its root system compared with species such as subterranean clover (Bolger et al. 2003), and lower soil water content (see below). In the case of phalaris-based pastures reduced inputs of fixed N during the pasture phase were likely to be a contributing factor (Dear et al. 1999). Certainly the trends in apparent N mineralisation by the different treatments were consistent with calculated amounts of N₂ fixation over the preceding pasture phase at the Wagga Wagga site which indicated that the pure lucerne sward had the lowest inputs of fixed N (20 kg N/ha), followed by mixtures of phalaris and subterranean clover (average 61 kg N/ha;

Hayes et al. 2017c). Similar to a previous study in a similar environment (Dear et al. 2004), grain harvest from wheat following a phalaris pasture had a lower protein content compared with pure legume pastures. The exception in the present study was the lucerne-only treatment.

The lucerne-only swards had the lowest plant available water in the surface 0.6 m at the end of the summer fallow, significantly lower even than where lucerne was sown in mixtures with subterranean clover or phalaris. Given that total pasture productivity in the pure lucerne sward was significantly lower than other lucerne-based swards at the Wagga site (See Table 6 in Hayes et al. 2017c) the drier soil in the present study most likely reflects reduced infiltration associated with lower levels of trash and higher levels of exposed bare soil (Hunt et al. 2016; Hayes et al. 2018). This is further supported by the finding that plant available water was generally greater in treatments that included the strongly fibrous-rooted perennial species, phalaris. It is possible that reduced water infiltration associated with reduced groundcover is the primary explanation for the reduced biomass yields of the lucerne-only swards but as soil water content was not recorded during the pasture phase further research is required to support this assertion.

There were measurable effects of the lucerne-only treatment on wheat performance. Pure lucerne rows fostered the fastest growth rate of wheat seedlings, and perhaps as a result, led to the lowest herbage N concentration in seedlings at 11 weeks post-emergence. This was despite the reduced levels of plant available water which likely indicates that incipient rainfall was adequate to meet requirements of young seedlings. The lowest N concentration of wheat herbage at anthesis was found in the lucerne-only treatment, and in the sampling location between pure lucerne rows, despite this being a location with one of the highest mineral N values in the surface 0.60 m depth when sampled in April. It is likely that the substantially drier soil profile under the lucerne-only swards contributed to this result reducing mineralisation of N and the ability of the more mature wheat plant to utilise soil N. This highlights the importance of including suitable companion species with lucerne in these drier cropping-zone environments (Boschma et al. 2010; Hayes et al. 2018).

Site and seasonal factors undoubtedly contributed to the responses observed in crop growth attributable to previous pasture treatments and sampling location relative to the original drill row. Different yield responses

might be anticipated at more limiting sites or under different seasonal conditions. For example, the soil pH across the site, although acidic, was unlikely to inhibit wheat growth¹ due to the low levels of toxic Mn^{2+} and Al^{3+} ions (Hayes et al. 2012, 2017b). The significant differences in plant-available S and K observed at the start of the cropping phase might have been expected to lead to differences in wheat yield in a soil type that was more limiting in these nutrients. The differences in available N observed due to previous pasture treatment might have led to even greater grain yield differences in a year where spring rainfall and yield potential of wheat was greater. Further research is required over a greater range of seasons and sites to confirm the magnitude of wheat yield responses that might be anticipated following these pasture comparisons.

Conclusion

This study establishes the enduring legacy of the original pasture drill row in phased pasture crop rotations on a Red Kandosol soil in the medium-rainfall cropping zone of south-eastern Australia. A grain yield increase of 11% was observed in wheat growing on the original pasture drill rows compared to plants grown on the inter-row area. At the current site, the pastures were in place for 27 months and removed with herbicides 7 months prior to the sowing of wheat crop. Differences in key soil chemical indicators attributable to the original pasture drill row, including mineral N, total C, and plant-available S and K were still evident following harvest of the first wheat crop. This is a biologically significant finding demonstrating the indirect effect of plants on soil chemistry given that no synthetic fertiliser was applied during the wheat growth period or the pasture phase. It highlights the constrained chemistry of soil in the pasture inter-row and suggests a need to reduce gaps between pasture plants in order to maximise the functionality of soil across a cropping landscape.

This experiment provides new insights into the mechanisms driving changes in pH due to annual and perennial species. Previous assertions that apparent reductions in soil pH under subterranean clover were

¹ Conversions to pH in $CaCl_2$ can be made with the following equation:

$$pH_{CaCl_2} = -0.1463 \times (pH_{KCl})^2 + (2.6982 \times pH_{KCl}) - 4.2403.$$

($R^2 = 0.98$; $n = 286$; Wagga Wagga Agricultural Institute soils laboratory, G.J. Poile unpub.).

predominantly attributable to leaching of mineral N are not consistent with the data generated by the current study which showed relatively little relationship between mineral N and soil pH. The results instead suggested that the difference may have been associated with an increase in pH close to the perennial plant which could simply reflect an increase of alkali plant residue in the soil sample. Further work is required to either confirm this result or resolve the apparent anomaly. Differences in crop yield at the plot level attributable to previous pasture treatment were generally explained by N availability that was greater in lucerne/subterranean clover swards, and less in phalaris/subterranean clover swards where inputs of biologically fixed N during the pasture phase were less. An unexpected finding was the relatively low apparent N mineralisation in the lucerne-only sward, attributable to the significantly drier soil profile to 0.6 m. This result is likely due to reduced soil cover compared to lucerne-based mixtures, reducing water infiltration during the pasture phase and/or the summer fallow following pasture removal and highlights the importance of suitable companion species to be grown with lucerne to maximise productivity and increase the resource-use efficiency of those pastures.

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Chapter 5

Contrasting soil microbial abundance and diversity on and between pasture drill rows in the third growing season after sowing

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Contrasting soil microbial abundance and diversity on and between pasture drill rows in the third growing season after sowing

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Abstract

Changed spatial configurations at sowing have been investigated as a strategy to minimize interspecific competition and improve the establishment and persistence of multi-species plantings in pastures, but the impact of this practice on the soil microbiome has received almost no previous research attention. Differences in populations of bacteria and fungi in the surface 10 cm of soil in the third year following pasture establishment were quantified using quantitative polymerase chain reaction and terminal restriction fragment length polymorphism methods. Populations were compared on, and between, drill rows sown to either the perennial grass phalaris (*Phalaris aquatica* L.), perennial legume lucerne (alfalfa; *Medicago sativa* L.) or the annual legume subterranean clover (*Trifolium subterraneum* L.). Results showed that soil microbial abundance and diversity were related to plant distribution across the field at the time of sampling and to soil chemical parameters including total carbon (C), mineral nitrogen (N), pH, and available phosphorus (P), potassium (K) and sulfur (S). Despite the 27-month lag since sowing, pasture species remained concentrated around the original drill row with very little colonization of the inter-row area. The abundance and diversity of bacterial and fungal populations were consistently greater under drill rows associated with higher total C concentrations in the surface soil compared with the inter-row areas. Our results showed that the pH and available nutrients were similar between the subterranean clover drill row and the inter-row, suggesting that soil microbial populations were not impacted directly by these soil fertility parameters, but rather were related to the presence or absence of plants. The abundance of bacteria and fungi were numerically lower under phalaris rows compared to rows sown to legumes. The richness and diversity of fungal populations were lowest between rows where lucerne was planted. Possible explanations for this observation include a lower C:N ratio of lucerne roots and/or a lack of fibrous roots at the soil surface compared to the other species, illustrating the influence of contrasting plant types on the soil microflora community. This study highlights the enduring legacy of the drill row on the spatial distribution of plants well into the pasture phase of a cropping rotation and discusses the opportunity to enhance the microbiome of cropping soils on a large scale during the pasture phase by increasing plant distribution across the landscape.

Introduction

Soil health and function are of key interest to farmers and their advisors who are seeking to improve the utilization of soil resources and to increase the resilience of production systems in the face of extreme and variable climatic events. Soil microbial communities are fundamental to soil health and function (Doran and Ziegl, 2000; Van Der Heijden *et al.*, 2008; Toju *et al.*, 2018) and it is therefore important to identify and implement management strategies that enhance the abundance and diversity of desirable microbial communities. Some of the recognized beneficial influences of microorganisms on plant growth and nutrition include the rhizobium-legume symbiosis for biological nitrogen fixation, mycorrhizal associations for phosphorus (P) uptake, disease suppression and improved soil structure (Gupta *et al.*, 2019b).

However, beyond these examples the dynamic relationships between plant and microbial communities in agricultural landscapes remains relatively poorly understood. It is clear that plant growth promotes microbial proliferation by providing a source of nourishment, e.g., carbon (C) as the energy source, and it is known that different plant species provide soil habitat conditions [C and nitrogen (N) concentrations] that are conducive to the proliferation of specific microbial communities (Osborne *et al.*, 2010; Thakur *et al.*, 2015; Tiemann *et al.*, 2015;

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Gupta *et al.*, 2019b). Therefore, plant diversity in grassland ecosystems has been speculated to increase soil microbial diversity and promote biological functions; especially in soils lower in organic matter, as high plant diversity may provide a greater supply and quality of resources to microorganisms (Johnson *et al.*, 2003; Loranger-Merciris *et al.*, 2006; Eisenhauer *et al.*, 2013). It is also known that particular microbial populations are advantageous for plant communities by facilitating organic matter decomposition, nutrient cycling and suppression of root diseases (Gupta *et al.*, 2019b). Plant species present in grassland systems such as lucerne, subterranean clover and phalaris are generally associated with microorganisms involved in symbiotic (legume-rhizobia) and non-symbiotic (grass-diazotrophic) N fixation and mycorrhizal associations. Microorganisms vary in terms of their response to varying quantities and types of biologically available C, other energy sources or nutrient substrates. For example, as per the currently accepted ecological classification scheme for soil bacteria, some taxa are more associated with greater labile C pools (copiotroph) such as in rhizosphere soil, whereas the taxa that dominate in soils with lower organic C availability are known as oligotrophic. It has been suggested that bacteria belonging to β - and γ -Proteobacteria and Bacteroidetes are considered copiotrophic whereas genera belonging to Acidobacteria are considered oligotrophic, that is, low in C turnover and high in C-sequestration potential (Fierer *et al.*, 2007; Trivedi *et al.*, 2016). Thus, changes in the balance between C turnover and retention through variation in microbial communities due to plant rhizosphere effects may be crucial because of its effects on soil fertility, structure and sustainable crop production.

Agricultural practices have large and often unintended impacts on soil microbial communities. Manipulation of the pasture drill row may be a convenient way to positively influence soil microbial communities and function in integrated crop/forage production systems as the drill row can impact large areas at a fine scale with relatively little cost and inconvenience to the farmer (Hayes *et al.*, 2017a). The creation of the drill row is typically preceded by significant disturbance to flora through tillage and/or applications of agri-chemicals. Ecologically, it is presumed that this disturbance would revert the soil microbiome to an early stage of succession (Crews *et al.*, 2016) after which it would progress along the successional gradient for as long as the management of that field permitted. In phased pasture-crop rotations common across south-eastern Australia, a 4–7 yr phase of continuous annual winter crop production is commonly broken by a 3–5 yr pasture phase that includes perennial and self-regenerating annual forage species (Kirkegaard *et al.*, 2011). The pasture phase represents an opportunity for succession in the microbiome due to the low level of disturbance that typically occurs. However, no study that we could find has previously examined the impact of phased pastures on the microbiome or explored the extent to which the pasture drill row might impact the microbiome.

This study is a first step in understanding the legacy of the initial pasture drill row and its impact on microbial abundance and diversity in the rhizosphere. It asks the question whether differences in soil biota attributable to the drill row can be observed in the third year after sowing and tests two hypotheses: (i) that the abundance and diversity of microbial populations varies according to whether a grass or a legume was initially sown in the drill row, and (ii) that the abundance and diversity of microbial populations is greater in the soil directly beneath the original drill row compared to the area between drill rows.

Materials and methods

A series of experiments were established to test the impact of alternative spatial configurations of mixed pasture swards on pasture productivity and persistence (Hayes *et al.*, 2017b). A subset of treatments from one experiment in that series was sampled to determine the extent and nature of differences in soil microbiology attributable to different pasture drill rows and is the focus of this paper.

Site and treatment details

The experiment was conducted at a field site near Wagga Wagga, NSW, Australia. The soil was classified as a red Kandosol (Isbell, 1996) and is described in greater detail by Conyers *et al.* (2018). Pastures were direct-drilled with a three-point linkage seeder fitted with narrow points and press wheels set at 250 mm spacings on May 30, 2012. The seeder was fitted with two distributing cones and eight sowing tynes and was modified in such a way that seeds of two pasture species could be delivered to alternate tynes from different cones. Plots were $6 \times 4 \text{ m}^2$, replicated three times. Approximately 150 kg ha^{-1} of Starter 15 fertilizer (14.7% N, 13% P, 12% S; Incitec Pivot Fertilizers, Melbourne) was broadcast across the experimental area immediately prior to sowing. Weeds were controlled during the 6 months prior to sowing using glyphosate as required.

Treatments sampled for soil microbiology included subterranean clover (*Trifolium subterraneum* L.) sown in every drill row (SC-only), subterranean clover and phalaris (harding grass; *Phalaris aquatica* L.) sown in alternate drill rows (Phal:SC) and subterranean clover and lucerne (alfalfa; *Medicago sativa* L.) also sown in alternate drill rows (Luc:SC). Cultivars and sowing rates of subterranean clover were identical in each treatment and included a mixture of three cultivars, Riverina, Coolamon and Bindoon, sown in equal proportions by weight at a total of 4 kg ha^{-1} of viable seed. That is, where subterranean clover was restricted to only half the drill rows, the number of seeds of that species placed in the designated drill rows was doubled compared to where subterranean clover was sown in every drill row in order to keep the weight of subterranean clover seed sown constant across treatments. Two cultivars of lucerne, Aurora and Genesis, were sown in equal proportions by weight at a total of 3 kg ha^{-1} of viable seed. Phalaris cv. Sirolan was also sown at 3 kg ha^{-1} . Legume seeds were inoculated with the commercial strain of rhizobia for lucerne (RRI 128) and subterranean clover (WSM 1325) by applying a peat media the day prior to sowing.

Plant frequency

The physical location of the sown species relative to the original drill row was quantified on July 14, 2014 with a quadrat with external dimensions of $0.5 \text{ m} \times 0.5 \text{ m}$, divided into 100 cells each $50 \text{ mm} \times 50 \text{ mm}$. The quadrat was placed parallel to the visible drill rows on a representative area of the plot after the herbage was clipped to a height of approximately 20 mm. The number of squares containing the base of a respective sown species was counted and calculated over the total quadrat as 'frequency (%)' (Lodge and Gleeson, 1984).

Soil sampling

Soil samples were taken twice on September 17, 2014, first for soil microbial abundance and composition, and secondly for soil chemical characteristics. For the first soil sampling, ten cores

were taken from each sampling location within each plot, either on or between the original pasture drill rows, using a foot-corer with 20 mm diameter sampling to 100 mm depth. As the initial pasture experiment was sown on a row spacing of 250 mm, samples taken from between the drill rows were approximately 125 mm from either original drill row. Samples were kept cool and transported immediately back to the laboratory, where roots were separated and discarded. The remaining soil was refrigerated at 4°C and shipped in an insulated storage box to the analytical laboratory for molecular (bacterial and fungal composition) analysis.

DNA extraction and quantification

DNA was extracted from 0.3 g (dry weight equivalent) samples of soil, in duplicate, using the PowerSoil DNA kit (www.qiagen.com) following the manufacturer's protocol. Mechanical disruption of the soil was accomplished by bead-beating using Fastprep (2×) (speed 5.5, 30 s; FP120; Q-biogene Inc., CA, USA) after which the final DNA extracts were eluted using 100 µL of warmed (60°C) C6, for 5 min, to maximize DNA yield, and the extracts were stored at -80°C. DNA extracts were also further cleaned using the MinElute 96 UF PCR Purification Kit (www.qiagen.com.au) and DNA eluted into nuclease free water.

All DNA samples were tested for purity using a NanoDrop ND1000 (Thermo Fischer Sci. Inc.) and the concentration of DNA against a DNA standard (λ -phage DNA; $R^2 = 0.98$) using the QuantiT PicoGreen dsDNA assay (Invitrogen, MA, USA). The final extracted DNA was diluted tenfold in molecular grade H₂O and 3 µL was used per 15 µL polymerase chain reaction (PCR) reactions. Abundances of total bacteria and fungi were quantified using group-specific primers that have been used extensively for analyzing soil bacterial communities and shown not to amplify mitochondrial/chloroplast DNA [bacteria: F968/R1378 (AACGCGAAGAACCTTAC/CGGTGTGTACAAGGCCCGGGAACG) (Smalla *et al.*, 2007) and fungi: FR1/FF390 (TTGGTCATTAGAGGAAGTAA/TTYGCTGYGTTCTTCATCG) (Vainio and Hantula, 2000)] based on reagents in the QuantiTect SYBR Green PCR kit (Qiagen, Vic., Australia). All PCR reactions were carried out on a Strategene Mxpro3000P qPCR system (Agilent, Vic., Australia). Quantitative-PCR (qPCR) was performed against standards. Standard curves for bacterial qPCR were generated using a pGEM-Teasy vector-based clone and the number of 16S rRNA gene copies was estimated, whereas for fungal qPCR, known amounts of culture DNA from *Rhizoctonia*, *Fusarium* and a *Trichoderma* isolate were combined to make a standard curve for the quantification of fungal DNA in the samples. Details of PCR conditions are described by Gupta *et al.* (2019a).

Analysis of microbial diversity

Bacterial and fungal community compositions were analyzed using the terminal restriction fragment length polymorphism (TRFLP) method. Bacterial TRFLP was conducted using universal primers 8F and 1520R; the forward primer was labeled with FAM and the reverse primer with HEX. PCR cycles consisted of 5 min at 95°C followed by 30 cycles of 30 s at 94°C, 60 s at 55°C and 120 s at 72°C, with a final extension of 10 min at 72°C. Analysis of fungal diversity using TRFLP was carried out using primers ITS 1F and ITS 4 (Gardes and Bruns, 1993), labeled with FAM and HEX, respectively. PCR cycles consisted of 5 min at 95°C followed by 35 cycles of 60 s at 94°C, 60 s at 56°C and 90 s at 72°C, with a

final extension of 10 min at 72°C. Bacterial gDNA controls included *Escherichia coli* and *Listeria* (non-pathogenic species) as positive controls.

Amplified DNA fragments for both bacteria and fungi were cleaned using the SureClean kit (Bioline Inc.) and DNA was re-suspended in 20 µL sterile water. Cleaned PCR product (8 µL) was then digested using restriction enzymes AluI and CfoI for 3 h at 37°C, and the reaction was stopped by heating to 65°C for 20 min.

Fungal community DNA was amplified from 14 ng of template DNA using the ITS1F.FAM forward and ITS4R.HEX reverse primers (Gardes and Bruns, 1993). PCR was carried out in a 35 µL total volume using 0.4 µM of primers, 0.2 mM of dNTPs, 1× PCR buffer (Qiagen, Australia), and 4 units of HotStarTaq DNA Polymerase (Qiagen, Australia). The reaction conditions were 94°C for 1 min (denaturation); 56°C, 1 min (annealing); 72°C, 1.5 min (extension) for 35 cycles using a thermocycler. In addition to the sample DNA, a negative control without DNA and positive controls with *Rhizoctonia*, *Saccharomyces* species and *Fusarium* culture DNA were included in every PCR run.

Terminal restriction fragments were separated using capillary separation (ABI 3730 DNA analyzer, Australian Genome Research Facility, Adelaide) with a LIZ500-250 size marker (ABI). Size and intensity data were interpreted using GeneMarker analysis software (SoftGenetics Inc.), with a minimum cut-off of 200 intensity units used to distinguish terminal restriction fragments from background noise.

Soil chemical analyses

Two soil coring tubes, each 25 mm in diameter, were inserted at each respective sampling location. Results from only the two surface depths, 0–5 and 5–10 cm, are reported here as they relate directly to the sampling for soil microbiology. Soils were air-dried in a fan forced dehydrating oven set at 40°C and passed through a 2 mm sieve. Coarse organic material and gravel too large to pass through the sieve were discarded from the sample. Soil pH was determined on a 1:5 soil:1 M KCl solution shaken for 1 h and measured at 20°C. The same extractant was used for the determination of mineral N (ammonia [NH₄⁺] plus nitrate [NO₃⁻]) concentration using a QuickChem® 8000 series flow injection analysis system. Total concentrations of soil C and N were determined by dry combustion (Leco, 1995) according to method 6B2b of Rayment and Lyons (2011). Colwell phosphorus and potassium (Colwell, 1963) as well as available sulfur (KCl-40; Blair *et al.*, 1991) were determined at a commercial laboratory (Incitec Pivot, Werribee).

Statistical analysis

Data collected from the subset of treatments reported in this paper were analyzed with an analysis of variance (ANOVA) using a complete randomized block model in Genstat (v18.1.0, VSN International Ltd.) with sampling location as the fixed term and replicate as the random term for each soil depth.

All the TRF data were analyzed for size and intensity using GeneMarker analysis software (SoftGenetics Inc.), using default settings for TRFLP analysis with a minimum threshold of 200 intensity units used to distinguish true TRFs from background noise. Band intensities (heights) were imported into Microsoft Excel and the relative abundance of a TRF in a TRFLP profile was calculated against total peak heights of all TRFs in the profile. All peaks with heights that were <0.5% of the total peak height were not included in further analyses.

Multivariate statistical analysis for community composition comparison and diversity indices calculation from TRFLP fragment data was performed using PRIMER-E (Primer 7; Clarke and Gorley, 2006). TRF abundance data were Hellinger transformed and Bray–Curtis dissimilarity matrices (+1) were constructed, statistical analyses performed, and diversity estimates calculated. Cluster analysis was performed with the Similarity Profile analysis (SIMPROF) test (Clarke *et al.*, 2008). Significant differences in community structure were tested for location, plant type and interaction models with permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) and analysis of similarity (ANOSIM; Clarke and Ainsworth, 1993). Canonical analysis on principle co-ordinates was performed for factor groups that were found significant with PERMANOVA only. For significant PERMANOVA results, the nature of the location-based differences and their relationships to soil physico-chemical properties (as predictor variables) were tested using distance-based linear model (distLM) analyses (Clarke and Gorley, 2006). For this, all specified factors were used to identify variables that explained significant ($P < 0.05$) amounts of variation in bacterial and fungal community structures. Heatmaps for relative abundances of TRFs were generated using Minitab vR2018a (Minitab Inc., PA, USA). Shannon diversity (H), Pielou's evenness (J), Margalef's richness (d), the number of individuals (N) and relative abundances of bacteria and fungi were analyzed with ANOVA using Genstat (v18.1.0, VSN International Ltd.).

Results

Pasture biomass and distribution of sown species

Pasture botanical composition and biomass for the experimental site was previously reported in Hayes *et al.* (2017b). Pasture biomass increased from 0.7–2.0 Mg DM ha⁻¹ in year 1 to 9.0–10.7 Mg DM ha⁻¹ in the third growing season after sowing. The Phal:SC sward was consistently more productive ($P < 0.05$) than the Luc:SC and SC-only swards in all but the final year where Phal:SC and SC-only swards had similar quantities of biomass. The proportion of unsown weeds was greatest in the subterranean clover sward compared to other treatments and although incrementally increasing with time in all treatments, never exceeded 16% in any treatment (Hayes *et al.*, 2017b).

In year 3, the distribution of all sown species remained concentrated close to the respective drill row established 2 years prior. The basal frequency (%) for subterranean clover in the SC-only treatment was calculated as the average of both drill rows sampled and was higher in the inter-row area compared with both perennial species (Table 1).

Soil chemical characteristics

There were significant ($P < 0.05$) differences in total C, mineral N and available phosphorus (P), potassium (K) and sulfur (S) between sampling locations in the surface 5 cm of soil (Table 2). The values of all parameters were generally lower in the inter-row area compared to those on the original pasture drill row. However, soil mineral N and available S and K were significantly lower on the subterranean clover drill rows compared with those planted to the perennial species, and statistically similar to the inter-row area. There was no significant treatment effect on soil pH in the surface 5 cm, but in the 5–10 cm depth either under subterranean clover drill rows or in the inter-row area

Table 1. Basal frequency (%; \pm standard error) of subterranean clover, phalaris or lucerne in winter of year 3 on the original drill rows compared to the inter-row area (approximately 125 mm from the original drill row)

Species	Drill row	Inter-row	Sample number
Lucerne	60 \pm 40	0	$n = 3$
Phalaris	50 \pm 10	23 \pm 8	$n = 3$
Subterranean clover	77 \pm 19	40 \pm 14	$n = 6$

between subterranean clover drill rows soil pH was generally lower compared to other sampling locations. Available K was also significantly higher under the phalaris and lucerne drill rows compared to other locations ($P < 0.05$), although differences were not as large as in the surface 50 mm (Table 2).

Soil microbiology

Soil bacteria were more abundant under pasture drill rows that were established over 2 years prior to sampling, compared to soil collected in the inter-row area. The abundance of bacteria under the phalaris drill row was less than that under either the lucerne or subterranean clover rows and was intermediate between the drill rows planted to legumes and areas between drill rows (Fig. 1A). Similarly, the abundance of soil fungi was greater under previous pasture drill rows compared to the inter-row area. It was again intermediate under the phalaris rows compared to most other drill rows and the inter-row area (Fig. 1B).

Bacterial species richness (Fig. 2A) and diversity (Fig. 2B) was generally greater on the original drill row compared to the inter-row area for both the phalaris and subterranean clover. However, no difference was found in species richness and diversity between the lucerne drill row and the corresponding inter-row area (Fig. 2A and B), despite the large observed change in bacterial abundance (Fig. 1A). Fungal species richness and diversity were also generally higher on the drill row compared to the inter-row area (Fig. 2C and D), although differences between treatments were not significant ($P = 0.08$) due to large error terms. Values for both parameters were substantially lower in the inter-row area of the Luc:SC treatment compared with all other locations.

Multivariate analysis of TRF data with edaphic variables as vectors clearly showed the vectors for the dissimilarity between on-row and inter-row areas. The proportion of each factor in explaining variation in bacterial and fungal community structure due to sampling location was explored with distLM analysis. For bacterial community structure, organic C, C:N ratio, S, NO₃⁻ and NH₄⁺ were the significant ($P < 0.001$) variables (Fig. 3A), whereas for the fungal community structure mineral N, C:N ratio, NH₄, S and K were the significant ($P < 0.05$) predictors (Fig. 3B). Total N and Colwell P had little impact on the composition of bacterial and fungal communities. Canonical analysis of principle ordination of relative abundances for TRFs, constrained by location and edaphic factors, showed significant variation in microbial community composition between on-row and between-row samples.

Results from heat maps depicted distinct fungal and bacterial communities in soil (Fig. 4). There were clear differences between samples taken from the drill row compared to those from the inter-row area for both groups of organisms. There was generally good agreement in samples taken from the subterranean clover drill rows across the different experimental treatments. Two distinct groups of bacteria were apparent, one more dominant on

Table 2. Total carbon (C), mineral nitrogen (N), pH, available phosphorus (P), potassium (K) and sulfur (S) in the 0–5 and 5–10 cm layers at different sampling locations in September 2014, relative to the pasture drill row established in May 2012

Sampling location	pH _{KCl}	Total C (%)	Mineral N (kg ha ⁻¹)	P–Colwell (mg kg ⁻¹)	K–Colwell (mg kg ⁻¹)	S–KCl40 (mg kg ⁻¹)
0–50 mm depth						
B/n Luc:Sub	5.49	1.90	4.5	33.7	413	4.7
B/n Phal:sub	5.29	1.88	3.4	38.0	423	5.2
B/n Sub	5.36	2.02	5.2	48.0	397	4.9
Luc row	5.80	2.63	15.5	39.0	660	10.5
Phal row	5.51	2.37	10.1	38.3	797	13.0
Sub row	4.86	2.35	8.8	29.8	359	7.3
<i>P</i> -value	0.097	<0.001	0.02	0.017	<0.001	<0.001
<i>l.s.d.</i> _{0.05}	–	0.266	4.46	9.39	75.4	2.76
50–100 mm depth						
B/n Luc:Sub	4.22	1.26	3.4	28.0	330	7.6
B/n Phal:sub	4.16	1.26	2.7	16.7	300	7.5
B/n Sub	3.92	1.21	2.5	43.3	320	8.3
Luc row	4.29	1.28	4.4	24.7	390	7.7
Phal row	4.19	1.26	3.0	29.3	383	6.6
Sub row	3.98	1.24	2.9	26.4	293	5.6
<i>P</i> -value	0.037	0.987	0.233	0.136	0.023	0.03
<i>l.s.d.</i> _{0.05}	0.229	–	–	–	61.4	1.77

B/n, between (i.e., the inter-row areas); Sub, subterranean clover; Luc, lucerne; Phal, phalaris.

the inter-row area while the other was more dominant on the drill row. The drill rows also conferred a greater diversity of fungal communities compared to the inter-row area, and the fungal communities sampled from the drill row appeared to differ according to the pasture species that were sown.

Discussion

The presence of a growing plant is known to make a significant impact on soil microbial communities both in terms of their abundances, composition and activity utilizing the enhanced supply of C and nutrients from root exudation and turnover (Kuz'yakov and Razavi, 2019). This single point-in-time study illustrates an unanticipated legacy of pasture drill rows established more than 2 years prior to sampling soil microbial populations. Despite the 27-month lag, pasture species remained concentrated around the original drill row where the majority of the root influence would be concentrated, undoubtedly driving results we observed in the microbiome as the microbial abundance and diversity was reduced in the inter-row area where plant frequency was lower.

High plant frequency in the drill row was associated with increased total C and increased mineral N in the surface 50 mm. The chemistry of surface soil in the subterranean clover drill row contrasted that of soil in the rows sown to the perennial species, phalaris and lucerne, by being lower in pH ($P = 0.09$) and lower in the available macronutrients P, K and S ($P < 0.01$). These differences were not reflected in the abundance of bacteria and fungi, which tended to be lower under the phalaris rows compared to either the lucerne or subterranean clover rows. This result suggests that microbial abundance and diversity are being driven more by the composition of the plant material which is

scarcely reflected in the standard soil chemistry analysis. Previous studies have documented changes in decomposition and mineralization rates between these contrasting species following the termination of the pasture phase (Bolger *et al.*, 2003; Angus *et al.*, 2006). However, few studies have examined the microbiome under living swards and we can therefore only speculate that factors such as C:N ratios, root architecture, root turnover or the presence of more recalcitrant components in the tissue, such as lignin, impacts the microbiome. The current study supports the first hypothesis that the abundance and diversity of microbial populations vary depending on whether a grass or a legume was initially sown in the drill row, which is consistent with previous reports of changes in the composition of microbial communities induced by the presence of N₂-fixing legumes (Osborne *et al.*, 2010). These observations highlight the importance of retaining pasture legumes in production systems for the benefit of soil microflora.

Fungal species richness and diversity were substantially lower between rows in the lucerne/subterranean clover treatment compared to most other sampling locations ($P < 0.05$). We postulate that this may be the result of lower fibrous root proliferation in the inter-row area at the soil surface, on account of the strongly tap-rooted lucerne being sown in every second drill row. Lucerne is the perennial legume species most commonly grown in phased rotations with crops in dryland environments across south-eastern Australia (Dear *et al.*, 2010; Kirkegaard *et al.*, 2011) largely due to its superior drought tolerance and capacity for more consistent inputs of biologically fixed N₂ than annual legumes across a range of environments (Peoples *et al.*, 2012). Despite these benefits, lucerne swards in this region are characteristically low in ground cover (Hayes *et al.*, 2018) due to the high

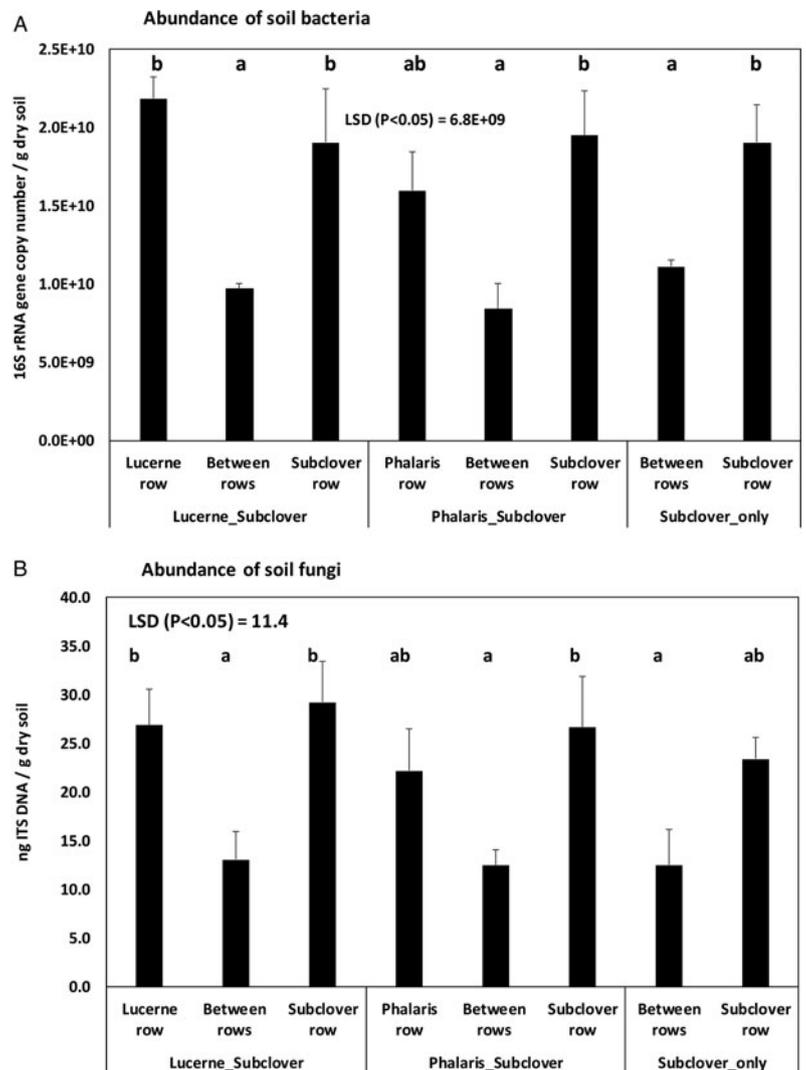


Fig. 1. Localized spatial variation between the pasture drill row and the inter-row area in the abundance of soil bacteria (A) and fungi (B) in pure subterranean clover swards (SC-only) and swards sown to subterranean clover and either phalaris or lucerne in alternate drill rows (adapted from Hayes *et al.*, 2017a). Bars marked with the same letter are not significantly different ($P > 0.05$).

degree of interspecific competition with subterranean clover (Dear and Cocks, 1997) and its superior capacity to exploit water in the soil profile to a much greater depth (Ridley *et al.*, 2001; Sandral *et al.*, 2006; Hayes *et al.*, 2010). The competitiveness of lucerne was a primary motivation for planting the two species in separate drill rows. Further research is required to determine the extent to which increased microbial diversity and abundance can be achieved in lucerne swards through a greater and more even distribution of fibrous-rooted species among the lucerne crowns and in particular, between drill rows.

There is little evidence that soil nutrition, within the range of values reported in this study, confers a direct impact on soil bacteria and fungi abundance and diversity. Rather, soil microbiology appears to be impacted primarily by the presence or absence of plants, as observed in the clear contrast in the microbiome under the pasture drill row compared to the sparsely populated inter-row area. It is noted that most of the responses in soil chemistry were plant-driven responses since fertilizer, for example, was not applied to the drill row and so changes in P, K and S are an indirect result of plant activity (see Table 2). We suggest that most of the differences in soil microbiology observed in the current study were also indirect and plant-driven.

The enduring legacy of the pasture drill row on the microbiome is an unanticipated but important finding. One might

have presumed that in the third year of a self-regenerating pasture, remnants of the original drill row would be scarce due to more uniform plant coverage across the field, especially for a self-regenerating annual species such as subterranean clover. On the contrary, present results demonstrated an enduring legacy in terms of plant distribution, which has resulted in significant shifts in soil chemistry and biology. It is possible that this result is due to the relatively low soil organic matter at this particular experimental site which potentially makes relatively subtle differences in the inter-row compared to the drill row more distinguishable. If we accept that a more resilient soil is one with a richer and more diverse microbiome, the implication of this research is that land managers need to find methods to increase the plant frequency across a field, especially during a pasture phase that is intended as a period of soil recovery in an otherwise intensive annual cropping program (Kirkegaard *et al.*, 2011; Angus *et al.*, 2015). Thus, the findings of this study suggest that greater diversity of plant species with contrasting habits, such as legume species vs non-legume species, as well as tap-rooted vs fibrous-rooted species, could positively influence the microbiome. In a recent study combining global observations with an experimental microcosm study, Delgado-Baquerizo *et al.* (2020) observed that soil biodiversity is significantly and positively associated with multiple ecosystem functions hence critical for maintaining soil

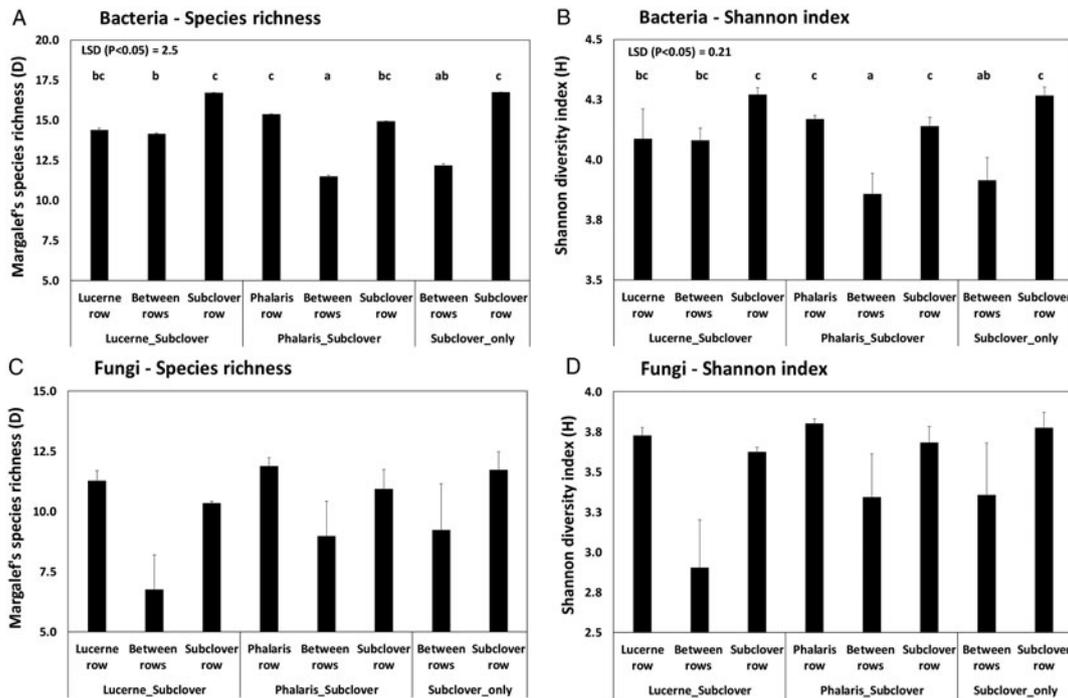


Fig. 2. Indices of species richness (Margalef; A and C) and diversity (Shannon; B and D) for soil bacteria (A and B) and fungi (C and D) sampled from the surface 100 mm in the third year after the establishment of drill rows sown to either subterranean clover, phalaris or lucerne, compared to soil sampled from the inter-row area. Bars marked with the same letter are not significantly different ($P > 0.05$); no bars marked with letters indicate that no significant differences exist between treatments ($P > 0.05$).

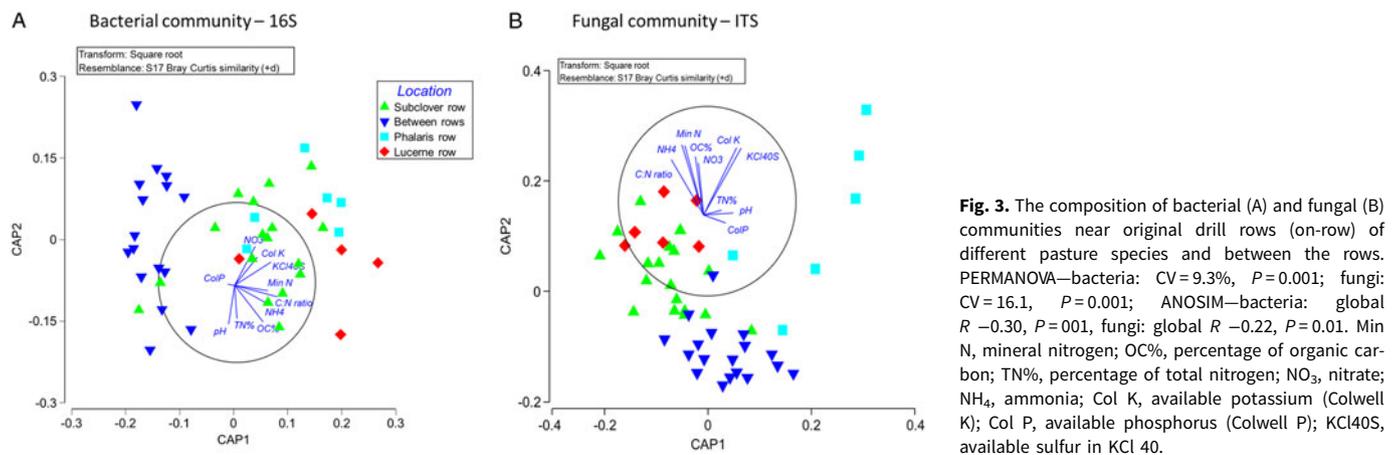


Fig. 3. The composition of bacterial (A) and fungal (B) communities near original drill rows (on-row) of different pasture species and between the rows. PERMANOVA—bacteria: CV = 9.3%, $P = 0.001$; fungi: CV = 16.1, $P = 0.001$; ANOSIM—bacteria: global $R = -0.30$, $P = 0.01$; fungi: global $R = -0.22$, $P = 0.01$. Min N, mineral nitrogen; OC%, percentage of organic carbon; TN%, percentage of total nitrogen; NO₃, nitrate; NH₄, ammonia; Col K, available potassium (Colwell K); Col P, available phosphorus (Colwell P); KCl40S, available sulfur in KCl 40.

functionality locally and across biomes. Increasing plant frequency in semi-arid environments such as those across southern Australia would likely need to be achieved without increasing plant density, as dense pasture stands are commonly not sustainable in such water-limiting environments. McCormick *et al.* (2014) quoted densities of 6–20 plants m⁻² as being adequate in a perennial species such as lucerne to fully utilize available resources (especially water), while Bowman *et al.* (2002) suggested that only 8 plants m⁻² might be required to contribute adequate amounts of fixed N to support subsequent cropping phase in semi-arid environments of south-eastern Australia. Such low densities present obvious challenges to increasing the frequency of plants across the landscape in order to facilitate a more abundant and diverse microbiome and speaks to the importance of

self-regenerating annual species to fill the gaps in such environments at times of the year when resources are more abundant.

Increasing plant frequency in the pasture phase to promote greater abundance and diversity of soil microorganisms across the cropping landscape will likely involve adopting narrower row spacings. Crop species grown at narrower row spacings generally achieve higher yields due to better utilization of available resources and less weed competition (Scott *et al.*, 2013). On the other hand, farmers wishing to reduce tillage frequency and stubble burning are forced to compromise and move toward wider row spacings to manage stubble loads. The benefits of minimum tillage cropping practices often outweigh grain yield reductions (Scott *et al.*, 2013). The same challenges exist when farmers are looking to establish a pasture in the year following a crop as the

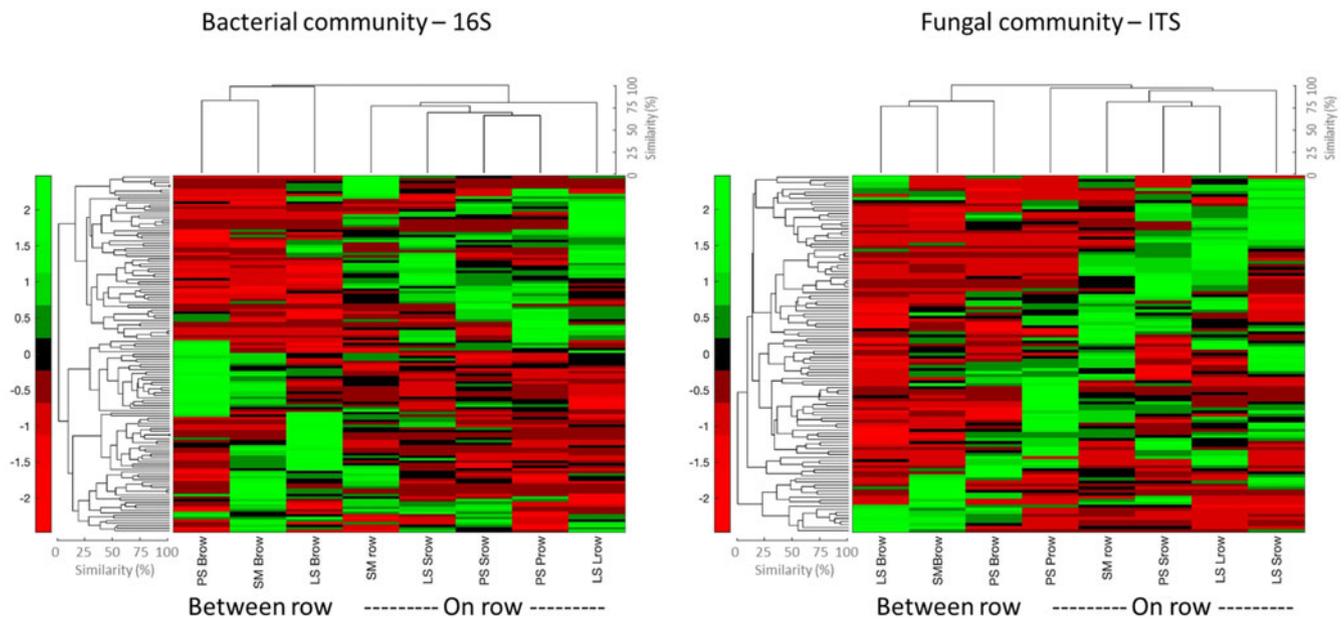


Fig. 4. Heat maps for the abundances of TRFs representing different soil bacterial (A) and fungal (B) communities as measured using TRFLP analysis. The color variation is presented for each TRF across different treatments (horizontal). Green color represents most abundant and red color represents least abundant. Horizontal cluster diagram represents percent similarity variation in the community composition between the different treatments. Vertical cluster diagram represents percent similarity variation between TRF groups. PS, phalaris-subterranean clover sward; SM, pure subterranean clover; LS, lucerne-subterranean clover sward; Srow, subterranean clover drill row; Prow, Phalaris drill row; Lrow, Lucerne drill row, Brow, between drill rows.

residual stubble burden will often not permit seeding equipment affixed with tynes at narrow spacings. However, on the basis of the enduring legacy of the pasture drill row that has been identified in this study, we postulate that yield reductions are potentially compounded over the life of the pasture, and the resilience of the cropping landscape could well be reduced, due to the greater inter-row area that we now know harbors a constrained microbiome.

Conclusion

The soil microbiome is fundamental to soil function and resilience. Increasing the abundance and diversity of microbial populations would seem to be a key strategy to maximize soil function and resilience. An obvious priority for future research would be to quantify the effects of the observed spatial heterogeneity in abundance and diversity of bacteria and fungi in terms of detailed phylogenetic and functional microbiome composition (using metagenomic techniques) and actual functions. Our study indicated that increasing plant frequency is the factor that most affects the abundance and diversity of both bacterial and fungal populations. Paying close attention to practices at establishment that would increase plant frequency offers great promise to significantly enhance the microbiome on a large scale across the cropping landscape. Achieving a higher plant frequency would seem to be particularly important in phased pastures where benefits compound over the life of the stand, and where legacy effects of the pasture phase have now been shown to be enduring.

The current study raises many additional questions that will require further research to better understand the potential impact of improving soil biology through increased plant frequency during the pasture phase. These include: (a) Can economic benefits of increased microbial diversity and abundance be quantified, perhaps through nutrient cycling or pest/disease management? (b) Are there environments or seasons in which increased microbial

diversity and abundance result in higher plant yields or persistence? (c) What other agronomic practices (e.g., changed crop rotations and multi-directional plantings) or mechanical modifications (e.g., scatter plates on seeders) might enable greater plant frequency to be achieved in short-duration pasture phases? In light of the unanticipated findings of the current study, we suggest that the microbiome in the pasture phase deserves greater research attention in order to increase the resilience of pasture/crop rotations.

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Chapter 6

Sowing configuration affects competition and persistence of lucerne (*Medicago sativa* L.) in mixed pasture swards

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Sowing configuration affects competition and persistence of lucerne (*Medicago sativa*) in mixed pasture swards

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Abstract. Spatial separation of species at sowing has been proposed as a means of managing interspecific competition in mixed swards. This study examined the effect of row configuration on persistence of lucerne (*Medicago sativa* L.) in pastures and pasture–cover crop mixtures at three sites in the Central West, and in pasture mixtures at three sites in the Riverina, New South Wales, Australia. Lucerne density, taproot diameter, groundcover, and spatial distribution relative to the original drill row were measured at all sites, and plant-available soil water and light interception during spring were assessed at some sites. Row configuration (alternate or mixed drill rows) did not affect lucerne persistence; however, where lucerne seed was concentrated in every third drill row, intraspecific competition led to increased lucerne mortality. This was estimated to occur at densities >28 plants/m drill row. A lucerne density of ~55 plants/m² in every or alternate drill rows (at row spacings of 250 mm) would likely achieve maximum lucerne production in the semi-arid environments tested, subject to the chance event of favourable conditions in the period after sowing that would maintain that density (e.g. cumulative summer rainfall >100 mm and summer day degrees <2160°C in 2 years at Cowra). The presence of a cover crop in the establishment year reduced lucerne density by 39% compared with pasture only, regardless of row configuration. Changed row configuration did not reduce competition for light under a cover crop, but there was a small increase in available soil water of up to 4.9 mm in the 0–1.15 m depth, mainly during the first summer, where pasture was sown in alternate compared with mixed drill rows with a cover crop. Soil was drier in pasture-only treatments than those with a cover crop, attributable to increased lucerne density and lower levels of litter cover on the soil surface. Pasture species remained largely confined to the original drill row, especially in drier environments, highlighting the importance of narrower row spacings for pasture establishment. In addition, we determined a mathematical relationship between lucerne density and the non-destructive measure of basal frequency; this relationship could be applied in mature lucerne stands with densities ≤80 plants/m².

Keywords: ground cover, soil water, alfalfa, cover crop.

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Introduction

Spatial separation of species at sowing is a management strategy that has been proposed as a means of reducing interspecific competition in mixed swards. The topic is of broad interest to agriculture globally and can be applied to a diverse range of crop and/or pasture species combinations (Butler *et al.* 2011; Bedoussac *et al.* 2015; Pembleton *et al.* 2016; Tautges *et al.* 2018). Work in south-eastern Australia has examined the practice in the contexts of increasing sward

diversity of pasture mixtures containing up to three species (Hayes *et al.* 2017b), and of improving pasture persistence when established alongside a vigorous cover crop (Hayes *et al.* 2017a). The rationale of spatially separating components at sowing is to reduce early interspecific competition between seedlings and allow all species better access to resources to support early development (Boschma *et al.* 2010; Butler *et al.* 2011). Presumably, as seedlings grow and increasingly access resources in the inter-row area both above- and below-ground,

the interspecific competition inevitably increases and the effectiveness of the original sowing configuration to reduce that competition may decline over time.

For perennial species such as lucerne (alfalfa, *Medicago sativa* L.) or self-regenerating annuals such as subterranean clover (*Trifolium subterraneum* L.), the legacy of the original drill row is poorly understood but is likely to affect species persistence and pasture productivity for the life of the pasture stand. For example, where a species is planted in every second drill row rather than every drill row, the effective row spacing of that species is doubled; this has been shown to have ongoing negative impacts on productivity, particularly for species such as lucerne with limited capacity to colonise the inter-row space during the life of the stand (Hayes *et al.* 2017a, 2017b).

Where a species is confined to a reduced number of drill rows, seeding rates can be adjusted to either a 'row' basis or an 'area' basis. Seeding on a row basis would keep the density of all drill rows constant for a given species, but the effective density of a species is reduced on an area basis as the number of rows containing that species is reduced. By contrast, seeding rates adjusted on an area basis will deliver the same quantity of seed of a particular species to a given area regardless of sowing configuration, although intraspecific competition within the drill row presumably increases as the number of seeds sown of a given species increases within a reduced number of drill rows. In mixed swards, calculation of seeding rates on an area basis is the most logical approach because the increase in intraspecific competition is somewhat offset by a reduction in interspecific competition through the exclusion of the other species. Calculation of seeding rates in mixed pasture swards on a row basis would reduce the density of all species in the mixture and risk negative impacts on yield (Palmer and Wynn-Williams 1976).

The present study examines the consequences of the spatial sowing configuration on lucerne persistence and tests several hypotheses: (i) lucerne persistence is affected by the initial sowing configuration; (ii) utilisation of resources such as light and soil water is reduced when pastures are confined to a reduced number of drill rows; (iii) the sown species remain largely confined to the original drill row in short-term pasture phases; and (iv) non-destructive measures of basal frequency do not accurately reflect lucerne density when lucerne is sown in alternate drill rows.

Materials and methods

Two series of field experiments were established to examine the effects of changed spatial sowing configuration on polycultures of pastures and/or crop mixtures in the Central West and Riverina regions of New South Wales (NSW). Six experiments in the Central West region compared mixtures of lucerne and subterranean clover established either in the presence or in the absence of a cover crop (harvested for grain at the end of the first growing season) in every drill row or confined to alternate drill rows. The second series of three experiments in the Riverina (south-west NSW) compared pasture treatments with lucerne grown in various spatial configurations with subterranean clover and/or the perennial grass, phalaris (*Phalaris aquatica* L.).

Central West region experiments

The six experiments were established in two consecutive years, 2013 and 2014, at three sites near Cowra (annual average rainfall (AAR) 619 mm), Bogan Gate (AAR 480 mm) and Condobolin (AAR 429 mm) and continued through 2016 (i.e. running for 3 or 4 years). The experiments were of split-plot design with crop type as the main-plot and spatial configuration as the subplots, with three replicates. Crop species included wheat (*Triticum aestivum* L. cv. Suntop), barley (*Hordeum vulgare* L. cv. Hindmarsh) and canola (*Brassica napus* L. cv. Stingray) at all three sites in both years, and lupin (*Lupinus angustifolius* L. cv. Mandelup) at Cowra in 2013 and at all sites in 2014. There were four spatial configuration treatments: Pasture-only, lucerne and subterranean clover seed sown in every drill row in the absence of a crop; Crop-only, the crop sown in every drill row in the absence of the pasture species but fallowed with herbicide in the year following grain harvest to prevent weed growth; Pasture-crop (mix), seed of lucerne, subterranean clover and the crop sown in every drill row; and Pasture-crop (1:1), seed of the crop sown separately to the lucerne and subterranean clover, in alternate rows. Plots were 12 m by 4 m in all experiments. Crop and pasture yield data are not reported here and are the subject of another paper (R. C. Hayes, M. T. Newell, A. D. Swan, M. B. Peoples, K. P. Pembleton, G. D. Li, unpubl. data).

All of the Central West experiments were in paddocks that had previously been cropped and chemically fallowed in the intervening time before sowing. Sowing rates are provided in Table 1. In 2013, the Cowra, Bogan Gate and Condobolin sites were sown on 17, 27 and 28 May, respectively. The 2014 experiments were sown immediately adjacent to the 2013 experiments at Bogan Gate, Condobolin and Cowra on 30 April, 1 May and 6 May, respectively. The seeder was fitted with two distributing cones connected to eight tines at a spacing of 0.25 m, fitted with narrow points and press wheels. Plots were sown with two passes of the seeder to achieve 16 drill rows per plot. Row configuration was manipulated by placing the seed of the crop into one distributing cone and the seed of the pasture into the other, then adjusting the tubes to control the tines to which the cones delivered seed of the respective species. Seeding rates of a particular species remained constant regardless of row configuration or the presence of companion species; when seed was confined to alternate drill rows (i.e. Pasture-crop (1:1)), the seeding density of the drill row was doubled, thereby maintaining the same overall seeding rate on an area (kg/ha) basis.

Prior to sowing, di-ammonium phosphate (75 kg/ha) and single superphosphate (75 kg/ha) were broadcast to the soil surface of each experimental area, providing 13.5 kg N, 22 kg P and 11 kg S/ha. In preparation for sowing, each site was sprayed with glyphosate (540 g/L, 1 L/ha) plus trifluralin (480 g/L, 2 L/ha), followed by an application of bifenthrin (250 g/L, 100 mL/ha) immediately after sowing. Once established, all sites received pinoxaden (50 g/L, 250 mL/ha) and flumetsulam (800 g/L, 25 g/ha) + bromoxynil (200 g/L, 700 mL/ha) in separate applications to control grass and broadleaf weeds. In subsequent years following establishment,

Table 1. List of treatments and sowing rates in the Central West and Riverina experiments, NSW
Locations were Cowra, Bogan Gate and Condobolin in the Central West, and Wagga Wagga, Eurongilly and Mirrool in the Riverina

ID	Treatment	Cultivar and sowing rate	Description
<i>Central West experiments (split-plot design; crop species × row configuration)</i>			
CW1	Pasture only	Cowra: lucerne cv. Pegasus (8 kg/ha) + subterranean clover cv. Coolamon (8 kg/ha); Bogan Gate and Condobolin: lucerne cv. Pegasus (3 kg/ha) + subterranean clover cvv. Seaton Park and Izmir (3 kg/ha each)	Lucerne and subterranean clover sown together in every drill row
CW2	Crop only	Cowra: wheat or barley (68 kg/ha), lupin (80 kg/ha) or canola (2.4 kg/ha); Bogan Gate and Condobolin: wheat or barley (23 kg/ha), lupin (40 kg/ha) or canola (0.6 kg/ha)	Respective crop species sown alone in every drill row
CW3	Pasture crop mix	As for CW1 and CW2	Respective crop species sown together with lucerne and subterranean clover in every drill row
CW4	Pasture–crop 1:1	As for CW1 and CW2	Respective crop in alternate drill rows to lucerne and subterranean clover
<i>Riverina experiments</i>			
R1	Lucerne only	Lucerne (3 kg/ha: 50% Aurora, 50% Genesis)	Lucerne sown in every drill row
R2	Lucerne:subterranean clover (1:1)	Lucerne (3 kg/ha: 50% Aurora, 50% Genesis) + subterranean clover (4 kg/ha in total comprising equal proportions by weight of cvv. Bindoon, Riverina, Coolamon at Wagga Wagga; cvv. Gosse, Goulburn and Coolamon at Eurongilly; cvv. Trikkala, Bindoon and Dalkeith at Mirrool)	Lucerne and subterranean clover sown in alternate drill rows
R3	Lucerne:subterranean clover (1:2)	As for R2	Two rows of subterranean clover to every row of lucerne
R4	Lucerne:subterranean clover mix	As for R2 and R3	Lucerne and subterranean clover sown together in every drill row
R5	Phalaris:lucerne (1:1) with subterranean clover	Sirolan phalaris (1.5 kg/ha) + lucerne (1.5 kg/ha: 50% Aurora, 50% Genesis) mixed with subterranean clover (4 kg/ha, cvv. as for R2)	Phalaris and lucerne sown in alternate drill rows; subterranean clover sown in every row
R6	Phalaris:lucerne (1:2) with subterranean clover	As for R5	Two rows of lucerne to every row of phalaris; subterranean clover sown in every row
R7	Phalaris:lucerne mix with subterranean clover	As for R5 and R6	Phalaris, lucerne and subterranean clover sown in every row

all pasture treatments received single superphosphate (150 kg/ha) with trace molybdenum (8.8% P, 11% S, 0.025% Mo) broadcast on the soil surface in autumn and were grazed by sheep periodically. The grazing regime is thought unlikely to have challenged the persistence of lucerne at these sites because there was generally a rest period of ~3 months between grazing events.

Riverina region experiments

Three experiments were established near Eurongilly (AAR 550 mm), Wagga Wagga (AAR 551 mm) and Mirrool (AAR 496 mm) in May 2012 as described in Hayes *et al.* (2017b). Seven lucerne-based treatments are reported here for the years 2012–14 (Table 1): lucerne only; lucerne–subterranean clover (1:1), (1:2) and mix; phalaris:lucerne (1:1), (1:2) and mix, all with subterranean clover.

Pastures were sown using the same seeder as described for the Central West experiments. Where a three-way mixture of lucerne, subterranean clover and phalaris was sown, seeds of the perennial species (lucerne and phalaris) were placed in different distributing cones and delivered to separate drill

rows, whereas subterranean clover seed was placed in both distributing cones and delivered to every drill row. Alternate row (1:1) treatments were achieved by condensing seed to only half of the drill rows, resulting in a doubling of seeding rate per drill row. In double-skip row (1:2) treatments where there were two rows of ‘species A’ followed by one row of ‘species B’, seed of species A was confined to every third drill row, whereas species B seed was confined to two rows in every three. Plots were 12 m by 4 m, except at the Wagga Wagga site where they were 6 m by 4 m.

Glyphosate (360 g/L) was applied up to three times at 2.0 L/ha to each experimental area in the previous spring, summer and autumn immediately before sowing, as required to control weed species. Starter fertiliser (14.7% N, 13% P, 12% S) was broadcast onto the soil surface at each site immediately before sowing. No other pesticides were applied to the experiments during the period of sampling. No grazing occurred at the Wagga Wagga site, which was instead mown and the material removed immediately following assessments of dry matter, at ~12-week intervals. The Eurongilly and Mirrool sites were grazed with sheep, also

at ~12-week intervals, similar to the grazing regime imposed at the Central West sites.

The key differences in experimental design between the Riverina and Central West experiments were that the Riverina experiments: (i) did not include mixtures with crop species, (ii) included the pasture species phalaris, (iii) included two skip-row (2:1) treatments, and (iv) had treatments where seed of lucerne and subterranean clover were planted in separate drill rows. However, there were many similarities in approach between the two series of experiments that make comparison of results across regions highly relevant. Most notably, all nine experiments were sown using the same seeder and tine assemblies, and the same 'area-based' approach to seeding was used, which meant that because species were confined to each drill row, the seeding density of a particular species increased proportionally (by 50%, 100% or 200%) within a drill row depending on the spatial sowing configuration compared with sowing in every drill row.

Weather data

Rainfall and temperature data were taken from the Australian Bureau of Meteorology (BOM). The research institutes at Wagga Wagga, Condobolin and Cowra are all registered BOM weather recording stations. For locations with no nearby observations of temperature (Mirrool, Eurongilly and Bogan Gate), interpolated daily maximum and minimum temperatures were sourced from the SILO gridded dataset (<https://www.longpaddock.qld.gov.au/silo/>; Jeffrey *et al.* 2001). Day degrees were calculated as the average of minimum and maximum daily temperatures and summed to give the cumulative day degrees for a given period.

Determination of plant location

In order to understand the relative distribution of the sown species at the end of the pasture phase and the extent to which their physical location shifted since sowing, their location relative to the original drill row was determined. Sampling occurred in July 2014 at the Riverina sites and September 2015 at the Central West sites, corresponding to the third year after establishment for the Riverina and 2013-sown Central West experiments, and the second year for 2014-sown Central West experiments.

In each plot, a representative area was selected and herbage from that area (0.75 m by 0.75 m) was cut to a height of ~10 mm with hand shears. A quadrat 0.5 m by 0.5 m, divided into 100 cells each 50 mm by 50 mm, was laid on the selected area so that 'columns' of cells within the quadrat ran parallel to the original drill rows, which were previously marked. The number of cells (of a maximum total of 10) within each column containing a live base of a sown species was recorded to calculate the relative frequency (%) of plants found on the initial drill rows compared with the frequency of plants found in the inter-row area or on 'other' drill rows to which that species was not initially sown.

Determination of lucerne density

Establishment density was assessed 2-4 months after sowing at each site by placing a 0.5 m ruler between two drill rows at random and counting seedling numbers on either side of the

ruler. This was repeated four times per plot and establishment density (plants/m²) was calculated as the sum of the 4 random counts. Accurate assessment of lucerne density beyond the year of establishment requires destructive sampling to distinguish individual taproots (Lodge and Gleeson 1984). Lucerne density was estimated on two occasions during the experimental period by excavating plants immediately after assessments of basal frequency, counting the number of individual taproots within the sample area and relating that to the measure of basal frequency. The first occasion, at all nine experiments, was immediately after the assessment of plant location (see above), when all lucerne plants in the quadrat were excavated by inserting a spade into the soil and excising taproots at a depth of ~150 mm. The diameter of each taproot was measured with callipers at ~10 mm below the lowest branch of the crown, there being a known association between lucerne taproot diameter and lucerne yield components (Suzuki 1991; Lamb *et al.* 2000; Ventroni *et al.* 2010; Hakl *et al.* 2018).

The second sampling occurred for the six experiments in the Central West only, and immediately followed a final assessment of basal frequency in 2016 with the purpose of estimating final relative density of mature lucerne plants. Briefly, two steel mesh quadrats (1.0 m by 1.0 m), divided into 100 cells, were placed in previously marked (fixed) locations in each plot, and the number of cells containing the live base of a lucerne plant was counted to determine basal frequency (Lodge and Gleeson 1984). Immediately following the assessment of basal frequency, the corners of the quadrats were marked with pin markers and the quadrat was removed. All lucerne plants within the fixed quadrat areas were excavated by inserting a spade into the soil and excising taproots at a depth of ~150 mm. The number of individual taproots was counted and recorded, but no assessment was made of taproot diameter.

Care was taken to ensure that destructive sampling did not impact on subsequent sampling. Often, such as at the second sampling of the Central West experiments, destructive sampling occurred at the conclusion of experiment, and no further assessments of the plots were taken. The earlier destructive sampling used a smaller quadrat to limit damage to plots. At the Wagga Wagga site in particular, where plots were smaller and the experiment was retained for further sampling beyond the pasture phase, sampling locations were marked and avoided at subsequent sampling times (Hayes *et al.* 2020a).

Light interception

Relative light interception was assessed on one occasion during September 2014 at all experiments except at Mirrool and the 2013-sown experiments at Cowra and Condobolin, which had insufficient herbage at the time of sampling. An SS1 SunScan Canopy Analysis System (Delta-T Devices, Cambridge, UK) was inserted below the canopy at ground level, perpendicular to the sowing direction (i.e. laid across the drill rows). In total, 64 sensors spaced at 15.6 mm over 1000 mm took readings of photosynthetically active radiation (PAR) from across a transect of the plot, which were averaged to provide a sample mean and compared with total PAR readings

taken concurrently from an above-canopy reference sensor (BF5 Sunshine Sensor; Delta-T Devices). Light interception was calculated as the difference between total PAR measured at the base station and average PAR at ground level across a transect of the plot. Values presented are the average of readings taken at four random locations per plot.

Groundcover

Groundcover was assessed in autumn of the second and third years after establishment (second year only for the three Central West experiments sown in 2014). Groundcover was visually distinguished into the following categories: plant parts attached to sown perennials; plant parts attached to (usually summer-growing) weeds, trash or litter (unattached plant materials); and bare ground (the inverse of total groundcover). Two methods were used to estimate groundcover. At the Riverina sites, a 'point' method was used in which the small area directly beneath the intersections of grids on a fixed quadrat in each plot was assigned one of the four cover types described above. The 0.5 m by 1.5 m fixed quadrat was divided into 0.1 m by 0.1 m grids, providing 96 intersections or points to assess. At the Central West sites, an 'area' method was applied to the fixed quadrats used for final assessment of basal frequency. All of the 0.1 m by 0.1 m cells of the 1.0 m by 1.0 m quadrats were examined, and only those cells with >50% of the area covered were counted under one of the four cover types described. The same operator assessed all plots on a given day in order to reduce error associated with subjective visual assessments.

Soil water

Owing to limited resources, readings of soil water were taken only at the 2013-sown Cowra experiment, using a neutron moisture meter (503 DR Hydroprobe; CPN California, USA). Aluminium access tubes (50 mm diameter) were inserted in the centre of each plot on 10 July 2013 by removing soil to a depth of ~1.25 m, considered to be the maximum likely rooting depth of crop species on this soil. Soil cores were subsampled by taking 30–50 mm of soil at each depth (0.15, 0.3, 0.45, 0.6, 0.75, 0.9 and 1.15 m), sealing in a polycarbonate jar with an air-tight lid, and transporting back to the laboratory. Fresh weight of each sample was taken before soil was oven-dried at 105°C for 5 days. The gravimetric moisture content (θ_g) was calculated as the difference in weight between fresh and oven-dried samples, adjusted for individual jar weight. Initial readings of the neutron moisture meter, taken on the same day that soil cores were removed, were related to θ_g values.

Two additional access tubes were inserted in two areas adjacent to the experiment to estimate field capacity and plant lower limit at that site. For determination of field capacity, a container (1000 L) was placed beside the sample area and filled with water. From the container, hose with drippers placed every 0.3 m was laid in concentric circles ~0.3 m apart in such a way that an area 4 m by 4 m surrounding one access tube was completely soaked. Black plastic was laid over the area for 3 weeks to minimise evaporation, and the container was refilled five times over the 3-week period. Once the container had emptied for the sixth time, the soil was left to drain for 48 h

before sampling. Three readings were taken with the neutron moisture meter at each of the above depths, and the soil immediately adjacent to the access tube was sampled in triplicate at each corresponding depth with a hydraulic soil corer and coring tubes (44 mm). Intact soil sections were carefully taken 50 mm either side (sections 100 mm) of the corresponding neutron moisture meter reading depth. Soil sections were sealed in polycarbonate jars and transported back to the laboratory. Soils were weighed, dried at 105°C for 5 days and weighed again to determine bulk density and θ_g . Volumetric moisture content (θ_v) was calculated for each sample as the product of bulk density and θ_g , and was assumed to be at field capacity for the samples taken from the irrigated area. Crop lower limit was determined in another additional area sown to lucerne at the same time that the experiment was sown. Soil was sampled on 25 February 2016 in triplicate by using a hydraulic soil corer as described above. Available soil water (ASW, mm) was calculated as the difference between field capacity and crop lower limit, adjusted for soil depth.

Statistical analyses

Data were analysed using GENSTAT 20th Edition (VSN international, Hemel Hempstead, UK). A REML-based meta-analysis was conducted for the final lucerne density (i.e. 2014 at Riverina and 2016 at Central West sites) and taproot data across all nine experiments. Fixed terms included site and row configuration with region as a factor to define the different experiments to which each data unit belongs, and replicate was included as a random term in the model. The Riverina sites were analysed independently from the Central West sites by using analysis of variance (ANOVA) for basal frequency, light interception and groundcover. Fixed terms were 'treatment', 'site' and treatment \times site for the Riverina experiments, and 'crop type', 'row configuration', 'site year' and all two- and three-way interactions for the Central West experiments. The random term was 'replicate' for the Riverina experiments, and replicate and replicate \times crop species for the Central West experiments.

The soil water data at the Cowra site were spline-fitted using a linear mixed model in ASReml-R (Butler 2020). The fixed factors were crop species, row configuration, sampling time and their interactions. Random factors were replicate, the spline component of sampling time, and associated interactions. All random terms were included in the initial model, but terms that failed to achieve statistical significance at $P = 0.05$ were excluded from the final model. The fixed effects were tested using the Wald statistical test, and the random effects were tested using the residual maximum likelihood ratio test.

Results

Seasonal conditions

Rainfall at the two drier sites in the Central West region, Bogan Gate and Condobolin, was generally close to average throughout the experimental period, except for 2016, which was substantially wetter than average at all sites. In 2013 and 2014, the Cowra site received very similar rainfall to the

Table 2. Monthly and annual total rainfall data (mm) for the Central West experimental sites compared with long-term average rainfall (LTAR)
Source: Australian Bureau of Meteorology

Year	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
<i>Bogan Gate</i>													
2013	27.6	55.2	79.8	0.0	23.2	127.8	50.0	19.6	53.6	10.2	13.8	35.0	495.8
2014	56.4	39.6	139.6	48.6	30.4	98.0	23.2	13.6	31.2	24.0	12.2	75.0	591.8
2015	55.0	3.4	1.0	72.4	29.4	24.2	85.6	40.2	13.2	11.4	50.2	33.8	419.8
2016	81.4	3.8	9.2	27.4	78.4	117.4	64.4	45.2	119.8	35.0	36.0	74.4	692.4
LTAR	28.9	49.4	50.2	28.7	32.4	49.8	37.8	32.7	39.0	39.1	45.5	52.3	479.9
<i>Condobolin</i>													
2013	5.9	17.6	47.8	0.3	25.2	124.1	38.8	10.0	47.4	7.7	3.4	18.2	346.4
2014	35.2	46.7	104.5	28.0	27.6	57.4	9.2	22.2	11.0	11.5	17.7	88.6	459.6
2015	59.2	35.9	0.2	64.7	11.6	31.8	41.2	42.3	6.8	65.2	67.3	28.5	454.7
2016	80.9	0.0	21.8	21.4	60.2	161.5	37.1	43.4	143.2	31.6	40.8	56.7	698.6
LTAR	29.2	33	26	17.8	26.5	27.2	33.1	29.4	23.6	34.2	32.8	25.8	428.7
<i>Cowra</i>													
2013	7.6	32.8	74.4	1.0	22.2	117.8	42.0	22.0	34.0	21.8	53.0	34.8	463.4
2014	11.2	50.4	102.6	49.8	33.6	55.4	34.2	25.8	33.6	34.2	17.4	72.0	520.2
2015	72.8	8.0	5.2	104.8	39.8	19.8	87.2	103.6	7.8	16.8	76.6	36.2	578.6
2016	68.2	3.6	28.4	34.6	71.2	130.0	111.0	38.8	163.2	84.0	43.4	57.2	833.6
LTAR	57.6	47.4	48.4	42.7	45.8	52.4	51.2	51.3	50.6	56.4	53.4	57.9	619.1

Bogan Gate and Condobolin sites; during these years, rainfall at the Cowra site was 25% and 16% below the long-term average (Table 2). The Cowra site had consistently lower cumulative summer temperatures than the Bogan Gate and Condobolin sites, but temperatures were higher at all sites in the 2013–14 summer than the following two years (Table 3). The Riverina sites all experienced above average February and March rainfall in 2012 before pastures were sown; however, the remainder of the experimental period was generally drier than average and characterised by a sequence of dry springs. Year 2 (2013) was the driest of the experimental period at all sites, with annual rainfall 22%, 27% and 29% below the long-term average at Eurongilly, Mirrool and Wagga Wagga, respectively (Table 4). The Eurongilly site was the coolest of the Riverina sites, and Mirrool the hottest (Table 3).

Plant location

There were significant ($P < 0.05$) main effects of site and row configuration on the final location of subterranean clover in the Central West experiments. A smaller number of subterranean clover plants was found on or close to (i.e. within 75 mm either side of) the original drill row at the two Cowra experiments sown in 2013 and 2014 (70.7% and 77.9%, respectively) than the experiments at Bogan Gate (88.5% and 94.4%) and Condobolin (90.9% and 92.8%) (l.s.d._{0.05} = 12.4). There were also significantly fewer plants on or close to the original drill row where pasture was planted in alternate rows with wheat (Pasture–crop (1:1), 72.6%) than where pasture was planted in every drill row with (94.3%) or without (90.8%) a wheat cover crop. There was no effect ($P > 0.05$) of site or row configuration on the final location of lucerne. Averaged across all treatments at all six of the Central West experiments sampled after 2 years (2014-sown

Table 3. Cumulative day degrees (°C) at the six experimental sites during the summer months of the experimental period
Data at Bogan Gate, Eurongilly and Mirrool were interpolated

Site	December	January	February ^A	Summer total
<i>Central West sites 2013–14</i>				
Bogan Gate	756	818	739	2313
Condobolin	767	843	760	2369
Cowra	698	785	695	2179
<i>Central West sites 2014–15</i>				
Bogan Gate	757	759	738	2255
Condobolin	772	770	757	2299
Cowra	719	732	685	2136
<i>Central West sites 2015–16</i>				
Bogan Gate	759	802	730	2292
Condobolin	781	821	742	2344
Cowra	708	757	685	2151
<i>Riverina sites 2012–13</i>				
Eurongilly	683	803	686	2172
Mirrool	724	842	715	2281
Wagga Wagga	699	822	707	2227
<i>Riverina sites 2013–14</i>				
Eurongilly	704	782	699	2185
Mirrool	733	813	728	2273
Wagga Wagga	722	797	720	2238

^ADoes not include 29 Feb.

experiments) or 3 years (2013-sown experiments), 99.4% (± 0.4) of lucerne plants ($n = 54$) remained on or close to the original drill row, whereas 85.9% (± 2.5) of subterranean clover plants ($n = 54$) remained on or close to the original drill row.

In the Riverina experiments in the third year after sowing, all species remained predominantly concentrated on or close to the original drill row. Across all sites, 99.5% (± 0.3) of

Table 4. Monthly and annual total rainfall data (mm) for the Riverina experimental sites compared with long-term average rainfall (LTAR)

Source: Australian Bureau of Meteorology

Year	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
<i>Eurongilly</i>													
2012	38.4	107.6	151.3	11.3	41.1	22.6	45.2	14.8	20.8	25.4	74.0	30.6	583.1
2013	19.0	83.4	25.0	7.4	39.7	88.4	53.3	42.1	35.6	14.4	7.0	16.4	431.7
2014	16.0	20.0	63.6	62.4	32.4	66.8	24.8	18.0	33.2	24.0	30.4	78.8	470.4
LTAR	43.2	42.7	38.9	43.6	46.0	44.7	50.9	47.3	47.3	52.8	46.9	45.8	550.1
<i>Mirrool</i>													
2012	81.0	111.4	125.2	7.4	43.6	30.8	42.2	41.2	9.0	12.8	47.3	17.4	569.3
2013	1.0	42.0	23.4	2.8	53.8	87.2	39.4	26.8	40.4	15.8	14.8	12.8	360.2
2014	20.0	25.6	40.0	53.0	32.2	69.4	27.4	16.0	34.8	16.4	21.6	26.8	383.2
LTAR	44.0	39.8	37.4	33.9	42.0	39.3	45.6	42.8	44.3	43.7	42.4	40.8	495.9
<i>Wagga Wagga</i>													
2012	72.4	50.8	193.4	8.0	41.8	17.1	42.4	26.8	6.0	17.7	35.8	48.6	560.8
2013	30.2	38.6	30.8	6.2	43.6	76.8	36.0	38.2	37.2	13.0	11.8	28.4	390.8
2014	13.2	27.2	63.4	56.6	47.0	81.0	24.2	10.6	36.6	22.0	47.0	29.0	457.8
LTAR	44.6	40.7	43.8	39.2	47.3	45.4	50.2	50.4	50.5	49.9	46.0	43.1	551.3

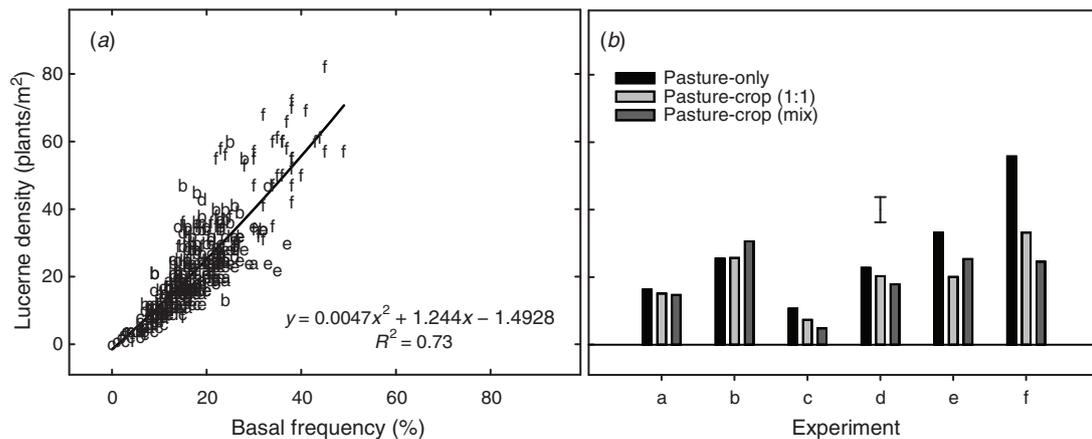


Fig. 1. (a) Relationship between basal frequency and lucerne density ($P < 0.001$, $n = 188$), and (b) site \times spatial configuration effects on final lucerne density across the six experiments in the Central West of NSW. In (b), data from individual experiments are marked according to letter: a, Bogan Gate sown 2013; b, Bogan Gate sown 2014; c, Condobolin sown 2013; d, Condobolin sown 2014; e, Cowra sown 2013; f, Cowra sown 2014. Error bar indicates l.s.d. at $P = 0.05$.

lucerne plants ($n = 63$) and 95.4% (± 0.9) of phalaris plants ($n = 45$) were found on or close to the drill row, whereas 82.8% (± 1.7) of subterranean clover plants ($n = 90$) remained on or close to the original drill row. There was a significant ($P < 0.05$) site effect on the final location of the subterranean clover in the Riverina experiments, with a higher number of subterranean clover plants located on or close to the drill row at the drier Mirrool site (92.7%) than the Wagga Wagga (74.6%) or Eurongilly (69.8%) sites ($\text{l.s.d.}_{0.05} = 5.5$). There was no effect of row configuration on the location of subterranean clover in the Riverina experiments ($P > 0.05$).

Lucerne density

In total, 8540 lucerne plants were excavated from 382 quadrats at the six experiments in the final year (2016) at the three Central West sites. The range in density of the quadrats sampled was 0–82 plants/m², compared with a range in

basal frequency of 0–49%. There was a strong correlation ($P < 0.001$, $R^2 = 0.73$, $n = 382$) between lucerne density and basal frequency (Fig. 1a). When all sites and treatments were included, basal frequency generally reflected lucerne density up to ~15 plants/m², but underestimated lucerne density by 20–25% in the range 15–30 plants/m² and by 25–30% in the range 30–80 plants/m². There was more error in the prediction of lucerne density from basal frequency in the Pasture-crop (1:1) treatments ($R^2 = 0.54$, $n = 128$), and density was underestimated above ~10 plants/m² ($y = 0.106x^2 + 1.2731x - 1.1555$). When the Pasture-crop (1:1) treatments were excluded from the analysis, the coefficient of variation increased to 80% and basal frequency closely reflected plant density up to ~20 plants/m² ($R^2 = 0.80$, $n = 254$, $y = 0.0086x^2 + 1.0964x - 1.5679$).

At each site, lucerne density was higher in the 2014-sown experiments, where stands were one year younger at the time

of sampling, than the 2013-sown experiments (Fig. 1b). Averaged across sites, lucerne density was 39% higher ($P < 0.05$) in the Pasture-only treatment (27.4 plants/m²) than either Pasture-crop treatment, that is, regardless of whether the crop was sown in mixed (19.7 plants/m²) or alternate rows (20.4 plants/m²) with the pasture. Averaged across sites, final lucerne density was significantly higher where it was sown in mixtures with lupins (30.1 plants/m²) than where it was sown with wheat, barley or canola (19.3, 20.7 and 22.3 plants/m², respectively). There was no significant ($P > 0.05$) crop × row configuration interaction.

Lucerne populations had generally declined to densities of 10–20 plants/m² after 4 years in the 2013-sown experiments, and to 20–30 plants after 3 years in the 2014-sown experiments in the Central West (Fig. 2). Lucerne densities generally remained higher in the Pasture-only treatments than treatments where wheat was sown with the pasture,

regardless of whether the wheat was sown in mixed or alternate drill rows, especially at the Cowra site. For the Riverina experiments, there was a significant effect ($P < 0.05$) of site and of row configuration on final lucerne density, but no significant site × row configuration interaction (Fig. 3). Of the three Riverina sites, lucerne density was always higher at Eurongilly, and although it declined at all sites during the experimental period, the fastest rate occurred at the Wagga Wagga site ($P < 0.05$). Initial lucerne densities were lower in the phalaris mixture treatments, reflecting the lower sowing rates, and there was no reduction in lucerne density in these treatments between the beginning and the end of the experiment. However, lucerne density did decline in the other treatments with time, especially in the Lucerne-subterranean clover (1:2) treatment, which confined lucerne to only one-third of the drill rows, where density declined to levels comparable with the lucerne-phalaris mixtures (Fig. 3b).

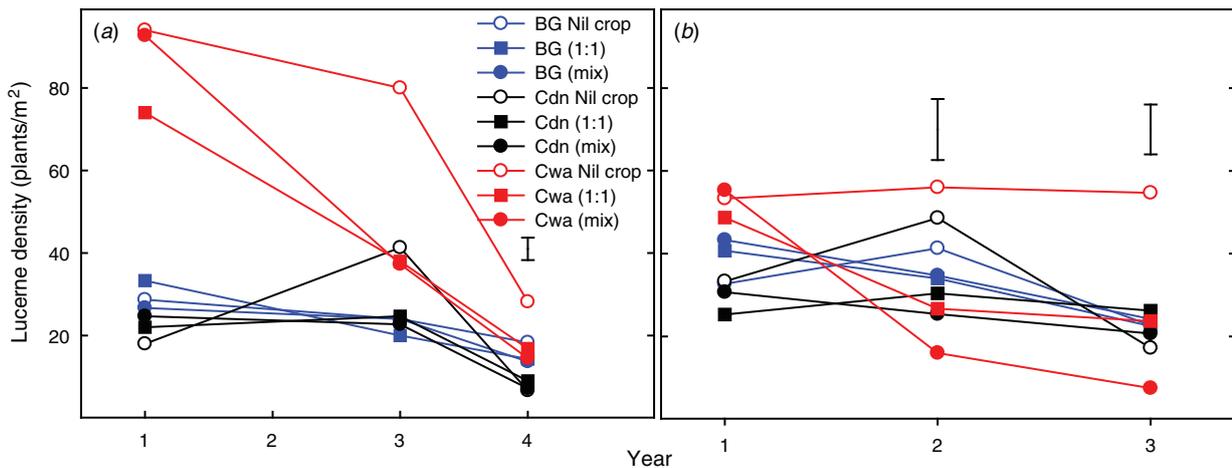


Fig. 2. Change in lucerne density through time due to spatial configuration associated with wheat crop at experiments sown at three sites in the Central West in (a) 2013, and (b) 2014. Error bars show 1 s.d. at $P = 0.05$ on dates where a significant site × treatment interaction was observed. BG, Bogan Gate; Cdn, Condobolin; Cwa, Cowra; Nil crop, Pasture only; 1:1, Pasture-crop (1:1); (mix), Pasture-crop (mix).

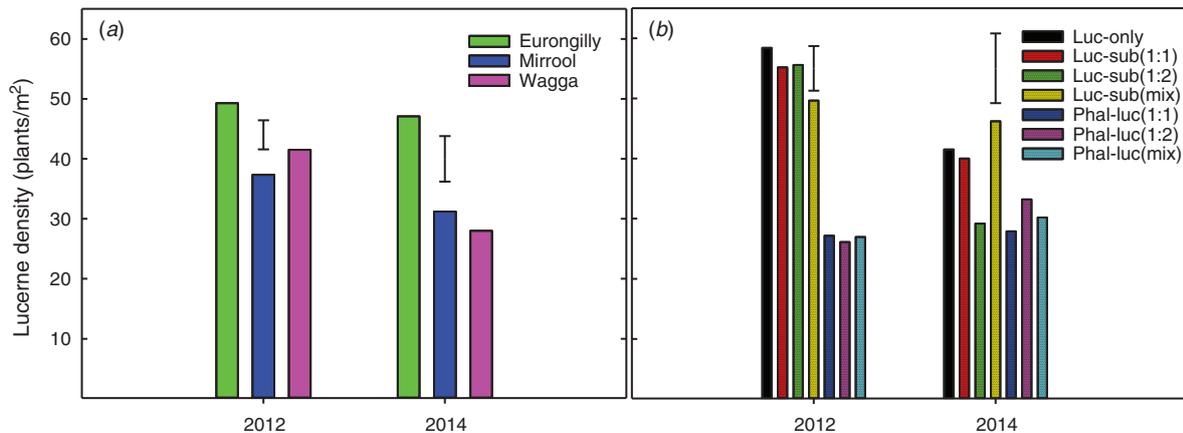


Fig. 3. Effect of (a) site, and (b) row configuration on lucerne density at the start (2012) and at the conclusion (2014) of the Riverina experiments. Error bars show 1 s.d. at $P = 0.05$. Luc, Lucerne only; Luc-sub, Lucerne-subterranean clover; Phal-luc, Phalaris-lucerne; (1:1), alternate drill rows; (1:2), lucerne every third drill row; mix, mixture in every drill row.

Lucerne taproot diameter

Lucerne taproot diameter was affected by site/year and by row configuration ($P < 0.05$) in both the Central West and Riverina experiments, but there were generally few significant site/year \times row configuration effects observed; therefore, only the main effects are presented in Table 5. Of the Central West experiments, taproot diameter was generally greater at the Cowra and Bogan Gate sites sown in 2013. There was no effect of row configuration on average diameter, although lucerne roots from the Wheat–pasture (mix) treatment had a greater minimum diameter, a narrower range in diameter, and a lower coefficient of variation than from other row configuration treatments. In the Riverina experiments, taproot diameter was greater at Eurongilly than the other sites. Again, there was no significant effect of row configuration on root diameter, but in the Lucerne-only treatment where average diameter was greater than in any other treatment, the minimum root diameter was also greater ($P = 0.07$) and the coefficient of variation lower ($P < 0.05$) than in other treatments. When data from both sites were combined, there was a weak, negative relationship between minimum root diameter and plant density ($P < 0.05$, $r^2 = 0.13$, $n = 117$) driven largely by a small group of samples at either extreme (i.e. either high plant numbers and small minimum root diameter readings, or the converse; data not shown). This correlation was improved ($r^2 = 0.25$) when plant density was expressed in terms of plant number per lucerne drill row.

Light interception

There were significant main effects of row configuration and site on light interception measured in the 2014-sown Central West experiments in spring of its first year, and a significant crop \times site interaction ($P < 0.001$). The Pasture-only treatment was able to intercept only 39% of PAR on the day of sampling, compared with 71% and 74% in the Pasture–crop (1:1) and Pasture–crop (mix) treatments, respectively (l.s.d._{0.05} = 4.79). Swards at Cowra intercepted a greater percentage of PAR (75%) than swards at Bogan Gate (65%), which in turn

intercepted more PAR than swards at Condobolin (51%). The significant crop \times site interaction was driven by a reduced interception of PAR in the lupin treatments at Cowra (Fig. 4a).

Table 5. Main effects of site/year and row configuration on taproot diameter (mm) of lucerne plants excavated from the Central West and Riverina experiments in 2014–15

Main effect	Average	Minimum	Maximum	Range	Coefficient of variation (%)
<i>Central West</i>					
Site					
Bogan Gate 2013	10.3	7.3	14.5	7.1	25.2
Bogan Gate 2014	6.6	4.1	9.9	5.9	28.4
Condobolin 2013	8.3	4.8	12.4	7.5	30.3
Condobolin 2014	8.2	4.3	14.4	10.1	36.8
Cowra 2013	10.6	5.6	18.4	12.8	35.3
Cowra 2014	7.6	4.8	11.1	6.3	31.4
l.s.d. ($P = 0.05$)	1.37	1.10	2.64	2.23	5.48
Row configuration					
Pasture only	8.5	5.0	13.4	8.5	31.6
Pasture–crop (1:1)	8.4	4.5	14.2	9.7	35.1
Pasture–crop (mix)	9.0	5.9	12.6	6.7	27.1
l.s.d. ($P = 0.05$)	–	0.78	–	1.58	3.88
<i>Riverina</i>					
Site					
Eurongilly	12.0	5.5	20.4	14.9	48.6
Mirrool	10.7	5.2	16.8	11.5	36.8
Wagga Wagga	10.6	6.6	17.1	11.4	36.6
l.s.d. ($P = 0.05$)	1.17	–	2.13	1.92	4.31
Row configuration					
Lucerne only	12.1	7.3	18.2	11.0	35.9
Luc-sub (1:1)	10.1	4.3	18.3	14.0	43.8
Lucerne–subterranean clover (1:2)	10.6	4.4	19.4	14.9	40.0
Lucerne–subterranean clover (mix)	11.5	6.5	18.1	11.6	38.6
Phalaris–lucerne (1:1)	11.1	5.4	17.2	11.7	36.7
Phalaris–lucerne (1:2)	10.8	5.9	18.6	14.6	47.6
Phalaris–lucerne (mix)	11.6	6.5	16.7	10.2	42.5
l.s.d. ($P = 0.05$)	–	($P = 0.07$)	–	2.93	6.59

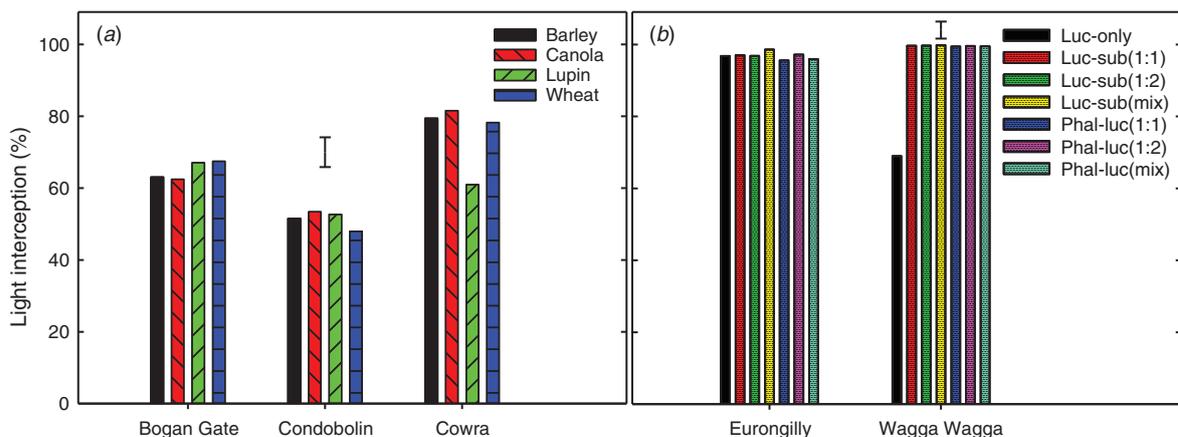


Fig. 4. Effect of (a) crop type at Central West sites (first year), and (b) pasture type at Riverina sites (third year) on light interception sampled in spring 2014. Error bars show l.s.d. at $P = 0.05$. Luc, Lucerne only; Luc-sub, Lucerne–subterranean clover; Phal-luc, Phalaris–lucerne; (1:1), alternate drill rows; (1:2), lucerne every third drill row; mix, mixture in every drill row.

The Bogan Gate experiment sown in 2013 was the only site to be assessed in its second year, and only the wheat-sown treatments were sampled because the crop species had been harvested 9 months before sampling. Similar to the first year measurements of the 2014-sown experiments, there was no difference in light interception between the Pasture-crop (mix) (45%) and Pasture-crop (1:1) (52%) treatments, but light capture in the Pasture-only treatment (41%) was again lower ($P < 0.001$, $\text{l.s.d.}_{0.05} = 10.8$).

There was a significant site \times pasture type interaction for light interception of pasture swards at the two Riverina sites sampled in their third year, Eurongilly and Wagga Wagga. All pastures at both sites were intercepting $>97\%$ of PAR at the time of sampling, except for the Lucerne-only treatment at Wagga Wagga, which was only able to capture $\sim 70\%$ of PAR (Fig. 4b).

Groundcover

There was a significant ($P < 0.05$) site \times row configuration effect on groundcover across all Central West experiments. Averaged across all sites, groundcover was $>78\%$ in all treatments except Pasture-only, which was significantly lower at 69%. Averaged across row configuration treatments, groundcover was higher ($P < 0.05$) at the 2013-sown Condobolin and both Cowra experiments (all 83%) than both Bogan Gate sites (74%), which themselves had higher groundcover than the 2014-sown Condobolin experiment (59%). The significant interaction was largely driven by the Cowra sites, where the difference in groundcover between the Pasture-only treatment and those containing crops was less than observed at the other sites. There was a significant negative correlation between litter cover (unattached plant material) and bare ground (the inverse of groundcover) across all six experiments in the Central West ($y = -0.848x + 83.81$, $r^2 = 0.70$, $n = 384$). This correlation was not as strong at levels of bare ground $<20\%$, mainly attributable to a group of data points from the 2013-sown Cowra experiment but not necessarily related to any particular row configuration treatment (Fig. 5b); when the Cowra-2013 site was excluded, the coefficient of determination increased to 91% ($n = 288$; Fig. 5a). Averaged across sites, litter cover was significantly higher in the Crop-only (71%) and Pasture-crop (mix) (70%) treatments than the Pasture-crop (1:1) treatment (65%), and lowest in the Pasture-only treatment (53%).

There were significant effects of pasture type, site and year of sampling on groundcover in the Riverina experiments ($P < 0.05$). At each site, groundcover was higher in the second than the third year, with the Eurongilly site showing the highest groundcover, followed by Wagga Wagga, then Mirrool. Averaged across sites and years of sampling, groundcover was significantly ($P < 0.05$) lower in the Lucerne-only treatment (54.8%) than the remaining six multi-species mixtures (range 64.9–66.7%; $\text{l.s.d.}_{0.05} = 4.59$) and there was no difference attributable to row configuration. This result was largely attributable to levels of litter, which were significantly lower in the Lucerne-only treatment (42.5%) than all other treatments (range 51.0–61.4%) except Phalaris-lucerne (mix) (45.8%, not significantly different from Lucerne-only).

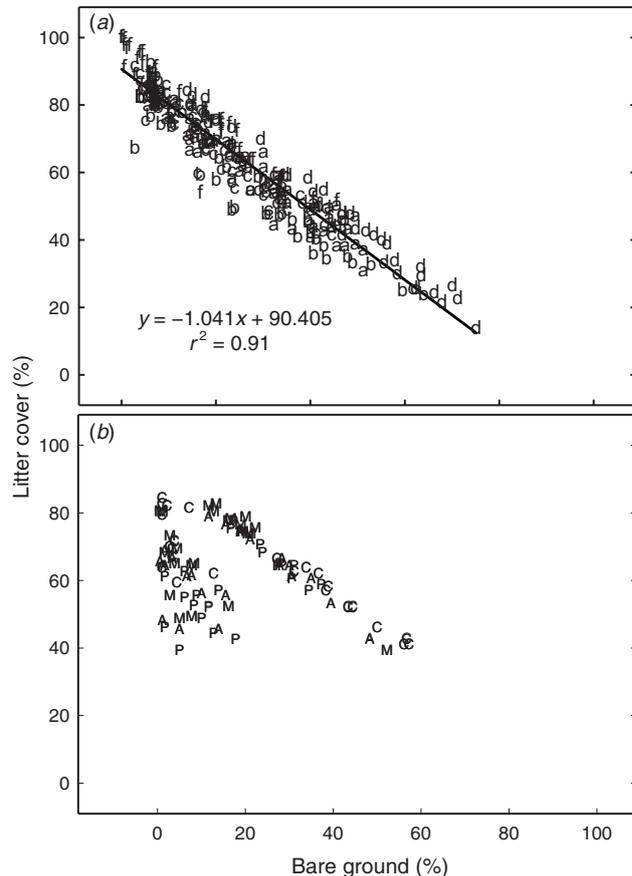


Fig. 5. Relationship between litter cover and bare ground at (a) all Central West sites except the 2013-sown Cowra site, and (b) the 2013-sown Cowra site. Abbreviations in (a) refer to site and year sown: a, Bogan Gate 2013; b, Bogan Gate 2014; c, Condobolin 2013; d, Condobolin 2014; f, Cowra 2013. Abbreviations in (b) refer to treatment: C, Crop only; P, Pasture only; A, Pasture-crop (1:1); M, Pasture-crop (mix).

Soil water

Significant effects of crop type and row configuration on soil water to 1.15 m depth at the 2013-sown Cowra experiment over time are shown in Table 6. Plant-available soil water was more abundant in the Crop-only plots, which were kept fallow from 2014, than the pasture plots. Among pasture treatments, soil was consistently drier under the Pasture-only treatment from the end of 2013 in the 0.75–1.15 m layer and tended also to be driest in this treatment in the 0–0.60 m layer, although differences in the surface layer were more dynamic, reflecting seasonal conditions. Soil was slightly drier in the Pasture-crop (mix) than the Pasture-crop (1:1) treatment in the period August 2013–April 2014, but thereafter, available soil water was almost identical under those treatments (Fig. 6). The difference in total available soil water in the 0–1.15 m depth between the Pasture-crop (mix) and Pasture-crop (1:1) treatments was no more than 4.9 mm, with the maximum difference observed in December 2013. By contrast, up to 14.7 mm additional soil water was available under the Pasture-crop (1:1) than the Pasture-only treatment to

Table 6. Wald statistics for main effects (crop types and row configuration) and their interactions for plant-available soil water from 10 July 2013 to 27 October 2015 in the 0.15–0.60 and 0.75–1.15 m depths and the whole soil profile (0.15–1.15 m) at the 2013-sown Cowra experiment
Effects: F, fixed; R, random. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s., not significant

Strata/decomposition	Effect	0.15–0.60 m	0.75–1.15 m	Whole profile
Experimental units				
Crop type	F	1.15n.s.	0.25n.s.	0.26n.s.
Row configuration	F	7.58*	26.18***	94.75**
Crop × row configuration	F	18.84***	27.76***	31.96***
Residual	R			
Experimental units × time				
Linear (time)	F	31.34***	376.30***	189.40***
Crop type × Linear (time)	F	0.56n.s.	1.24n.s.	0.33n.s.
Row configuration × Linear (time)	F	0.92n.s.	38.83***	11.45***
Crop type × row configuration × linear (time)	F	0.59n.s.	0.61n.s.	0.50n.s.
Spline (time)	R	***	***	***
Crop type × spline (time)	R	n.s.	n.s.	n.s.
Row configuration × spline (time)	R	***	***	***
Crop type × row configuration × spline (time)	R	n.s.	n.s.	n.s.
Residual	R			

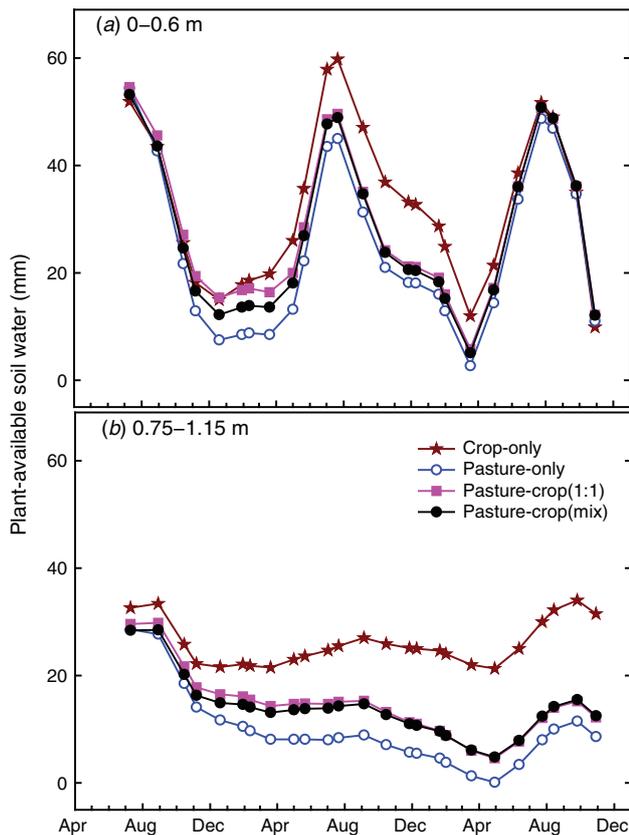


Fig. 6. Effect of spatial configuration on plant-available soil water at (a) 0–0.60 m and (b) 0.75–1.15 m depths at the Cowra site from July 2013 to October 2015.

1.15 m depth, with the maximum difference observed in March 2014.

Discussion

This study examined the consequence of the spatial sowing configuration on lucerne persistence at nine field experiments. The following sections deal with each hypothesis in turn.

Lucerne persistence

There was little evidence in the present study that lucerne persistence was affected by sowing in alternate drill rows compared with sowing in every row. However, density was reduced where lucerne was confined to every third row (Lucerne–subterranean clover (1:2)) compared with planting in every or alternate drill rows. Lucerne mortality increased by 37% in the Lucerne–subterranean clover (1:2) treatment compared with the Lucerne–subterranean clover (mix), a difference ascribed to intraspecific competition, because no difference in density was observed between those treatments shortly after sowing. At the three Riverina sites, average establishment density was ~55 plants/m². This equates to ~14 plants/m drill row where the lucerne was sown in every row, 28 plants/m drill row where lucerne was sown in alternate drill rows, and 37 plants/m drill row in the Lucerne–subterranean clover (1:2) treatment. Apparently, a density of 37 lucerne plants/m drill row was too high to be sustained at these sites despite similar plant densities among treatments on an area basis. Therefore, the first hypothesis of the study is accepted that lucerne persistence is affected by the initial sowing configuration, with the caveat that persistence was reduced only when lucerne density within the drill row exceeded an apparent maximum threshold, defined by site and seasonal conditions that were broadly similar for the three Riverina environments tested.

It is interesting that the final density of lucerne in the Lucerne–subterranean clover (1:2) treatment was very similar to densities observed in the Phalaris–lucerne treatments (~30 plants/m²) where lucerne was sown at only half the rate when expressed on an area basis. However, when expressed in terms of number of plants per length of drill row, final lucerne density ranged from only 8 plants/m in the Phalaris–lucerne mix treatment to 22 plants/m in the Lucerne–subterranean clover (1:2) treatment. This suggests that densities presented on an area basis are a more reliable measure than those presented based on number per length of drill row, and highlights the fact that in mature swards, competition exists at a scale beyond the drill row. It is difficult to account for effects of interspecific competition

on lucerne from the phalaris and subterranean clover; numbers of plants of those species were not recorded because it is difficult to distinguish individuals. Nevertheless, the fact that lucerne density of all swards declined to 30 (± 3) plants/m² regardless of the differences in productivity and composition of these swards is significant and suggests that lucerne persistence was impacted more by intraspecific than by interspecific competition at these sites. This probably reflects the winter-growing nature of both companion species and the dominant influence of the summer period in determining lucerne persistence in these semi-arid environments (Bowman *et al.* 2002).

Many previous studies have reported that after the establishment year, lucerne densities move toward a common lower limit (Christian 1977), with mortality shown to be density-dependent (Palmer and Wynn-Williams 1976). Initial densities in the present study were <100 plants/m² regardless of site. In the Central West experiments, second year densities were generally 25–50 plants/m², third year densities 20–30 plants/m², and fourth year densities 10–20 plants/m². Third year densities at the Riverina experiments were generally 40–45 plants/m² except in the treatments mentioned above where lucerne seeding rates were lower, which were ~30 plants/m². The Cowra site stood in contrast, with the Pasture-only treatment retaining higher densities than all other treatments and sites in the Central West, especially in the 2014-sown experiment where there was no apparent decline in density over the 3 years. This could reflect the lack of competition with a cover crop in the first year compared with the Pasture-crop treatments at that site, combined with relatively favourable conditions over the duration of the experiment allowing >50 plants/m² to be maintained. It seems unlikely that the higher seeding rate at the Cowra site explains the improved persistence of lucerne compared with Condobolin and Bogan Gate. As densities declined at Bogan Gate and Condobolin beyond the second year, increasing seeding rates at those sites would likely have served simply to increase the rate of density decline early in the pasture phase.

Lucerne density at the 2013-sown Condobolin site had declined to <10 plants/m² after 4 years, which is similar to densities previously suggested (8–10 plants/m²) as being minimum thresholds for adequate lucerne production in semi-arid environments (Bowman *et al.* 2002; McCormick *et al.* 2014). Wolfe and Southwood (1980) proposed that 10 lucerne plants/m² would be adequate for the Wagga Wagga environment provided other companion species were sown, such as subterranean clover. In semi-arid environments where lucerne survival is frequently challenged by moisture stress and high soil temperatures (Bowman *et al.* 2002), there is an ongoing compromise with lucerne swards. On the one hand, in the range of densities commonly found in arid environments, lucerne herbage production is proportional to stand density (Latta and Blacklow 2001). On the other hand, lucerne mortality increases at higher densities (Palmer and Wynn-Williams 1976), particularly under moisture stress. Increased density by increasing seeding rates in such environments would seem to be an advantage only by chance, should seasonal conditions following establishment be favourable.

Favourable seasonal conditions can explain the contrasting pattern in lucerne decline observed at the 2014 Cowra experiment compared with the 2013 experiment (see Fig. 2). Negligible lucerne mortality was recorded in the Pasture-only treatment at the Cowra site over two consecutive summers, 2014–15 and 2015–16, associated with cumulative rainfall >100 mm and cumulative day degrees for the 3 months <2160°C. Increased rainfall alone does not explain the reduced lucerne mortality; both the Bogan Gate and Condobolin sites received ≥ 100 mm rainfall in each summer but were substantially hotter, recording day degrees >2250°C, likely explaining increased lucerne mortality at those sites.

Increasing seeding rates in semi-arid environments increases the cost of sowing and risks initial establishment densities that are above the threshold of sustainability at a given location. Yet, there is an opportunity cost of lost herbage production (and reduced inputs of fixed nitrogen; Peoples *et al.* 2012) if the initial establishment density is below the maximum threshold defined by the soil and seasonal conditions. The maximum threshold, linked to future seasonal conditions, is inevitably unknown; hence, there would seem to be merit in higher rather than lower seeding rates to mitigate the risk of lost lucerne production early in the life of the stand (Latta and Blacklow 2001; Swan *et al.* 2014). Seeding rates were not directly compared in the present study. However, we can calculate that the upper limit of lucerne density sustained in the Riverina environments was between 28 and 37 plants/m drill row. At 37 plants/m drill row, intraspecific competition led to increased lucerne mortality. We could argue that the upper limit is closer to 28 than to 37 plants/m drill row on account of a smaller minimum taproot diameter observed at the lower density (in the 1:1 row configuration treatments), perhaps suggesting that, at this density, a larger number of plants are more vulnerable to mortality (see further discussion of this point below). Therefore, if we assume that 28 plants/m drill row is the upper limit of density and assume a similar strike rate (seedlings surviving as a proportion of number of seeds sown) as achieved in the present experiments, we can conclude that a sowing rate of lucerne ~6 kg/ha in these environments will provide maximum lucerne production without increasing mortality due to intraspecific competition. This suggests that the lucerne sowing rates used at the Riverina sites (1.5–3 kg/ha) were too low for maximum lucerne production, with present recommendations of lucerne seeding density being '1–5 kg/ha, depending on rainfall' (Lattimore and McCormick 2012). Stated differently, present commercial lucerne seeding rates are probably targeted more at reducing lucerne mortality rates beyond the first year than on maximising lucerne production.

In drier environments such as at Condobolin or Mirrool, lucerne sowing rates that approach 6 kg/ha might be excessive, because the probability of receiving favourable conditions following sowing to sustain high plant populations is remote. For more favourable locations such as Cowra or Eurongilly, those sowing rates seem both realistic and desirable. Further research is necessary to categorise more objectively particular locations and ascribe an

appropriate lucerne sowing rate based on relative climatic risk. Our study suggests that the productivity of lucerne-based pastures in drier regions could be improved not by focusing on the lucerne component, which inevitably will be limited by soil water resources during the hot and dry summers frequently experienced in the Central West and Riverina environments, but by focusing more on the winter-growing component of the sward. Increasing the productivity of the annual legume or even the perennial grass (Hayes *et al.* 2017b) had relatively little impact on lucerne survival in our study.

Competition for light and water

At the Riverina sites, there was no difference in lucerne density between the Lucerne-only and Lucerne-subterranean clover treatments, other than the Lucerne-subterranean clover (1:2) as discussed previously. Again, this speaks to the relatively minor effect of interspecific competition from winter-growing forage species on lucerne persistence. Minimum taproot diameter was greater in the Lucerne-only treatment ($P = 0.07$) than all other treatments, yet by the third year, this treatment was less productive than other multi-species treatments (Hayes *et al.* 2017b). This was most evident at the Wagga Wagga site, demonstrating an apparent inefficiency in production compared with Lucerne-subterranean clover treatments, which achieved higher biomass yield with similar lucerne densities, attributable to the contribution of subterranean clover. In this regard, lucerne and subterranean clover in these environments were shown to be compatible species. This inefficiency was highlighted by the substantially lower levels of light interception in the Lucerne-only sward at the Wagga Wagga site and is suggested in a companion paper to be associated with lower levels of plant-available water in the profile (Hayes *et al.* 2020a), although soil water was not measured until after the end of the pasture phase. Reduced soil water in this treatment could be a result of lower levels of groundcover, which is known to enhance infiltration on similar soil types (Hunt *et al.* 2016), and was likely exacerbated at the Wagga Wagga site by a subsoil constraint associated with an aeolian layer at ~1.2 m, inhibiting access to soil water below that depth (Hayes *et al.* 2010).

In the Central West experiments, the range in taproot diameter was greater in the Pasture-crop (1:1) treatment owing to a smaller minimum taproot diameter than in the Pasture-crop (mix) and Pasture-only treatments, despite there being no difference in average diameter among treatments. We could find no studies that directly link taproot diameter with lucerne persistence. However, taproot diameter has previously been associated with lucerne yield components (Suzuki 1991; Lamb *et al.* 2000; Ventroni *et al.* 2010; Hakl *et al.* 2018) and yield components are associated with persistence (Christian 1977). Therefore, it is logical that reduced taproot diameter might expose lucerne plants to greater risk of mortality, particularly under elevated abiotic stress. More specifically, crown diameter has been linked to persistence, with lucerne plants having larger crowns shown to be more persistent, particularly under grazing (Brummer and Bouton 1991). Crown diameter is likely to be correlated with taproot

diameter, although few studies have directly measured both traits.

Santhirasegaram and Black (1967) identified competition for light as the primary explanation for reduced pasture yields under a wheat cover crop. This is consistent with observations from the present study that showed Pasture-only treatments able to capture just <40% of PAR in spring of the establishment year, compared with >70% where pasture was sown with a cover crop. This undoubtedly reflects the lower vigour of pasture species compared with the crop species tested. There was no difference in apparent light interception between the Pasture-crop (mix) and Pasture-crop (1:1) treatments, although this may simply reflect the timing of sampling; a more detailed assessment over time may have shown differences earlier in the development of the crops and pastures. Nevertheless, it was of little consequence to lucerne persistence between the two treatments, and it seems that the alternate spatial arrangement (on a 250 mm drill row spacing) was not an effective strategy for reducing competition for light by a cover crop in the crucial spring period of the establishment year. An unexpected finding was that at the 2013-sown experiment at Bogan Gate, there was still no difference in apparent light interception between the mix and 1:1 treatments some 9 months after the crop was harvested, and both still had marginally higher interception than the Pasture-only treatment. The likely explanation was that the standing stubble in those Pasture-crop treatments was still shading the sensors on the light meter, and likely shading the pastures growing beneath them. The extent to which competition for light is a driving factor governing production in later years of a pasture phase remains unclear, but it seems that pastures with a 500 mm spacing between pasture drill rows (i.e. the Pasture-crop (1:1) swards) will struggle to achieve complete canopy cover without significant colonisation of the inter-row area.

The soil water data from the Cowra site sown in 2013 provide insight into how the changed row configuration impacts competition for water. The Pasture-only treatment was drier to 1.15 m depth than either the Pasture-crop (1:1) or Pasture-crop (mix) treatments. This result undoubtedly reflects the greater density of lucerne in the Pasture-only treatment than in the pastures that were established under a cover crop, but could also be associated with lower groundcover, which has long been known to restrict water capture through lower rates of infiltration (Glendening 1942; Hunt *et al.* 2016). This could be due to either increased runoff or increased evaporation on these plots, and we speculate that increased evaporation is more likely, given the relatively flat topography at that site. Despite crops being harvested for grain at the end of the establishment year, their stubbles were retained and slowly broke down over the remainder of the pasture phase, leading to greater levels of litter on the soil surface. Infiltration rate was not measured in the present study, but it should not be ruled out as a contributing factor to the drier soil observed in the Pasture-only treatment. Many previous experiments across this region have examined soil water dynamics under perennial-based pasture species and attributed differences to the drying capacity of the species present (Ridley *et al.* 2001; Sandral *et al.* 2006; Hayes *et al.*

2010, 2016). However, few have examined litter cover and the possibility of low levels of cover under some perennial swards reducing water infiltration and confounding results. For example, in all of the studies listed, soil was consistently found to be drier under lucerne than perennial grasses, but it is also common for groundcover to be significantly lower under lucerne than under perennial grasses such as phalaris (Hayes *et al.* 2018). This is a priority area of future research because increasing litter cover, either through the strategic use of cover crops or use of well adapted companion forage species, appears to offer great promise for increasing the productivity of pastures grown in semi-arid environments via the conservation of soil water.

The apparent increase in available soil water in the Pasture–crop (1:1) compared with the Pasture–crop (mix) swards suggests that the 1:1 row configuration was effective in managing competition for water in the 0–1.15 m depth of soil, for a short period at least. The timing of this difference (late 2013–early 2014) aligns with when the crop in each alternate row was likely to have stopped using water and before the pasture in every other row had time to explore the crop drill row for resources. After April 2014, not only would the lucerne have had time to develop lateral roots to explore the zone previously occupied by the cover crop, but the subterranean clover would also have been regenerating and exploring the soil for resources. In this instance, litter cover does not explain the result because there was significantly more litter cover on the drier Pasture–crop (mix) treatment. Therefore, we partially accept the second hypothesis that the utilisation of resources such as water is marginally reduced when pastures are confined to a reduced number of drill rows, but there was little difference in light interception attributable to row configuration.

Location relative to the original drill row

The third hypothesis of the study was accepted: that sown species remained largely confined to or within 75 mm either side of the original drill row. This was especially true for lucerne, where >99% of plants remained on, or close to, the original drill row at the end of the pasture phase. A lower percentage, although still a majority, of subterranean clover plants also remained on, or close to, the original drill row. The greater propensity of subterranean clover than of lucerne to spread away from the initial drill row (short-distance dispersal) undoubtedly reflects its annual growth habit and regeneration from seed. We observed a greater proportion of subterranean clover plants in the inter-row area at sites where rainfall was generally more favourable, such as Cowra or Eurongilly, than at drier sites such as Mirrool or Condobolin. In the Central West experiments, we observed a greater proportion of subterranean clover plants in the inter-row area where it was sown (with lucerne) in alternate row configurations with a cover crop, compared with plots where pasture species were sown in every drill row. Both observations suggest that the ability of subterranean clover to spread is a function of water availability. At the drier sites, the furrow created at sowing probably offered ongoing benefits in terms of water capture and infiltration, which helped to concentrate

subsequent regeneration close to the original furrow. Where pasture was sown in a 1:1 configuration with a cover crop, increased water resources were available after the first year in the previous crop drill row, due to the absence of lucerne or crop plants.

Another obvious factor affecting the ability of subterranean clover to spread after the first year is the physical location of seed or burr, which, in part, is governed by the size of plants the previous year and by soil conditions at seedset. The length of stems, branches and peduncles, and the number of inflorescences, govern where seed and burr are set relative to the original crown (Donald 1954). Presumably, a greater number of inflorescences set further from the crown assist in the short-distance dispersal of subterranean clover. However, increased seedset is expected from burr that is buried compared with burr remaining on the soil surface and is likely to occur more frequently for the earliest formed burrs owing to more favourable soil conditions (Rossiter 1961). Earlier formed burrs are inevitably located closer to the crown.

The implication for short-term pastures, particularly in drier environments, is that plants will remain concentrated on the original pasture drill row. This has been shown to have implications for spatial variability in soil fertility (Hayes *et al.* 2020a), soil biology (Hayes *et al.* 2020b) and may also affect groundcover and productivity. Better outcomes in all of these parameters might be anticipated from sowing at narrower, rather than wider, row spacings.

Lucerne density and frequency

The relationship between lucerne density and basal frequency is important, because basal frequency is often used as a non-destructive measure for monitoring plant density over time (Lodge and Gleeson 1984). Although basal frequency is used increasingly in studies of lucerne swards, few studies have examined the nature of the relationship between frequency and density. Vogel and Masters (2001) describe the importance of the frequency measure as a cheap and easy tool for monitoring plant density and suggest that frequency counts taken with 0.75 m by 0.75 m quadrats divided into 0.15 m by 0.15 m cells overestimate plant density, and that values should therefore be multiplied by 0.4 to provide a ‘conservative’ approximation of density. However, they provided no evidence of a calibration equation that validates their coefficient for calculating density, apparently assumed the same relationship for all species, and almost certainly did not consider the possible consequences for changed spatial configuration of species. Lodge and Gleeson (1984) used a 1.0 m by 1.0 m quadrat divided into 0.1 m by 0.1 m cells and studied the relationship in lucerne swards of varying density. They found that this method accurately reflected lucerne density in the range 20–80 plants/m²; the function that they fitted had a coefficient of determination of 99.1%. By contrast, the quadratic function fitted to the relationship in the present study had a coefficient of variation of only 77% and overestimated density by up to 30% in the range 15–82 plants/m². The apparent anomaly is likely explained by three factors. First, Lodge and Gleeson (1984) studied a larger range of densities, approaching 500 plants/m², and fitted one function across the full range.

Second, we have many more data points ($n = 382$) in a tighter range ($0\text{--}82$ plants/m²), which inevitably adds to the error and explains the reduced coefficient of variation. Third, many of our data points ($n = 128$) were taken from plots where lucerne was sown only in alternate rows. Estimates of lucerne density from basal frequency in these swards were considerably less accurate ($R^2 = 0.54$) than in swards where lucerne was sown in every drill row ($R^2 = 0.80$). Therefore, we accepted our final hypothesis that non-destructive measures of basal frequency do not accurately reflect lucerne density when lucerne is sown only in alternate drill rows.

Conclusions and practical recommendations

Spatial sowing configuration had no effect on lucerne persistence in alternate row (1:1) configuration compared with in every drill row. However, where lucerne was confined to every third row, there was an increase in mortality attributable to intraspecific competition at lucerne densities >28 plants/m drill row. We calculate that a lucerne sowing rate of ~ 6 kg/ha, when delivered to every drill row at row spacings of 250 mm, would likely achieve maximum lucerne production in the semi-arid environments tested, but is subject to the chance receiving favourable conditions in the period after sowing to maintain adequate lucerne densities. Favourable conditions at the Cowra site included cumulative summer rainfall ≥ 100 mm and cumulative summer day degrees $<2160^\circ\text{C}$. At drier environments where the frequency of favourable seasonal conditions is likely to be lower, we reason that more emphasis should be placed on the winter-growing species as opposed to the lucerne component of the sward to improve productivity, because interspecific competition from winter-growing forage species had little impact on lucerne persistence.

By contrast, interspecific competition from vigorous cover crops established in the first year consistently increased lucerne mortality compared with plots where pastures were sown without a cover crop. Alternate row configurations were ineffective at reducing competition for light in the establishment year. However, this configuration was shown to provide a marginal increase in soil water compared with sowing pasture in every drill row, an effect observed only for several months after the crop had ceased growing. Regardless of row configuration, final lucerne density was, on average, 39% higher where it was sown without a cover crop than where a cover crop was sown.

All sown species remained largely confined to the area close to the original drill row for the period of experimentation, up to 4 years. Short-distance dispersal was more evident in subterranean clover, on account of its annual growth habit, than in lucerne, and occurred more at sites where seasonal conditions were more favourable. The implication of this result is that short-distance dispersal is less likely to occur at drier sites, increasing the importance of narrower row spacings for pasture establishment.

The study established two mathematical relationships that will have future application. The first is a quadratic function that can be used to predict lucerne density from non-destructive measures of basal frequency. Valid in the range

$0\text{--}80$ plants/m², this will have utility in dryland lucerne swards after the establishment year, which rarely exceed that density. The relationship between basal frequency and lucerne density was not as strong when lucerne was sown in alternate rows compared with every drill row. The second is a negative relationship between litter (or trash) cover and exposed bare soil. For assessments of groundcover in perennial-based pasture swards taken in late summer–early autumn there is often little cover afforded by live plants. Cover instead is driven largely by unattached plant matter that can arise from crop or pasture residues. Pure lucerne pastures were found to have lower levels of litter cover than mixtures that included companion crop or pasture species, and this was implicated as a contributing cause of drier soils under some lucerne-based pastures. Examining practical means to increase the litter cover of lucerne swards through companion species or cover crops seems a worthwhile line of future research.

Conflicts of interest

The authors declare no conflicts of interest.

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Chapter 7

Consequences of changing spatial configuration at sowing in the transitions between crop and pasture phases

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Consequences of changing spatial configuration at sowing in the transitions between crop and pasture phases

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Abstract

Prospects for improving the productivity of species mixtures by changing row configuration at sowing was examined in two series of field experiments in rainfed, semi-arid environments in south-eastern Australia. The first series explored how the choice of cover crop and use of alternate drill row configurations affected establishment and yields of sward components in the transition from a cropping into a pasture phase. Crop species included wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), canola (*Brassica napus*) and lupins (*Lupinus angustifolius*), each sown in mixed or alternate drill rows with lucerne (*Medicago sativa*) and subterranean clover (*Trifolium subterraneum*). The second series of experiments examined the productivity implications when returning from the pasture phase back into cropping following 10 contrasting pastures sown to various combinations of lucerne, subterranean clover, or phalaris (*Phalaris aquatica*) sown in mixed, alternate (1:1) or double skip (1:2) row configurations.

In the transition into the pasture phase, the alternate drill row configuration reduced grain yields from cover crops by an average of 24% compared to the mixed drill row treatment. The presence of pastures had little impact on crop yields. Canola and lupin crop biomass were lower, compared with wheat and barley, which fostered increased pasture yields in year 1 associated with reduced lucerne mortality at some sites. Pasture yields beyond year 1 were reduced by ~20% in the alternate drill row treatments compared to where species were sown in mixed drill rows, with the yield differences increasing under more favourable growing conditions. This response largely reflected yield reductions in lucerne where it was concentrated in fewer drill rows. The alternate drill row configuration increased the abundance of subterranean clover in year 1, but this species remained a minor component of all swards beyond the establishment year.

In the transition into the cropping phase, grain yield was only affected by row configuration of the previous pastures to the extent that row configuration changed pasture composition. Grain yield and quality reflected the productivity and legume composition of the previous pastures but varied across sites. Whereas the 1:1 treatment provided advantages to the productivity of the self-regenerating subterranean clover, no such benefits were observed in the perennial species, lucerne. The impacts on total yields depended on the dominance hierarchy of species, which varied with site. Taken together, our results highlight the opportunity of changed row configuration to improve subterranean clover yields. The importance of minimising row spacing is discussed.

Keywords

Alfalfa, lucerne, subterranean clover, phalaris, cover cropping, establishment

Introduction

Pastures grown in rotations with crops in the mixed farming regions of southern Australia enhance crop yields by providing a pest and disease break (Kirkegaard *et al.* 2008) and through improvements in soil structure and fertility often attributed to nitrogen (N) inputs from perennial or self-regenerating annual legumes (McCallum *et al.* 2004, Peoples *et al.* 2012). The transitions into and out of the pasture phase are critically important for the management of productive and efficient grain/livestock production enterprises. The transition between phases involves elevated complexity that needs to be managed; a particular challenge in countries such as Australia where enterprises are typically on a large scale and farm labour is increasingly limited (Bell and Moore 2012). Invariably, good management and

an attention to detail in the transition between phases will impact the productivity and profitability of crops or pastures in following years. For example, a high weed burden at the start of a pasture or crop phase will likely remain in subsequent years suppressing growth and yields; low density of sown species at establishment will likely remain low throughout the pasture phase.

The transition from crops to pastures has long been a topic of research interest. Pasture species that are broadly adapted to the mixed farming regions of southern Australia, such as lucerne (alfalfa; *Medicago sativa* L.), are typically slow to establish, requiring farmers to take land out of production temporarily to allow seedlings to develop before they are grazed. Cover cropping, the planting of pasture seed concurrently with seed of the final crop in the rotation, has long been used as a means of managing this transition to reduce the period that land is effectively out of production (Santhirasegaram and Black 1967, McCormick *et al.* 2014). It is less expensive to under-sow lucerne with the final crop compared to sowing it alone the following year but pasture composition is almost always compromised with weed incursion consistently greater in pastures that are established under a cover crop compared to those established alone (Li *et al.* 2014, Swan *et al.* 2014). Nordblom *et al.* (2017) concluded that on farms running higher livestock densities, sowing lucerne alone rather than with a cover crop would likely lead to lower financial risk for farmers. However, they acknowledge that substantial year-to-year variation in financial outcomes associated with uncorrelated variability in seasonal conditions and commodity markets, along with other factors such as farm indebtedness, often masks or reduces the financial advantage of sowing lucerne alone. Together with the fact that previous analyses have scarcely valued some benefits that a cover crop may afford a pasture in some environments through increased cover, such as protection from wind (Blanco-Canqui *et al.* 2015), it can reasonably be assumed that many farmers will continue with the practice of establishing pastures under cover crops. This raises the question, how might crop and pasture yields be optimised in a cover-crop scenario?

One approach is to spatially separate crop and pasture species in drill rows at sowing to limit competition and ensure crop and pasture components both have access to resources for early development. This approach is increasingly being used in perennial crop research as a means of managing competition between the crop and companion species (Hayes *et al.* 2017b, Dick *et al.* 2018, Tautges *et al.* 2018). Some legume species, such as subterranean clover, appear to benefit from being spatially distant from companion species at sowing (Hayes *et al.* 2017a, Hayes *et al.* 2021b), with pasture biomass increased by up to 128% compared to where it was sown together in the same drill row with an early-generation perennial wheat (Hayes *et al.*

2017b). However, similar to the experience in Minnesota USA (Tautges *et al.* 2018), Hayes *et al.* (2017b) demonstrated a significant decrease in grain yields compared to a pure cereal treatment. These results contrast the one study from Canada where the inclusion of legumes between drill rows sown to the perennial crop, Kernza[®], *Thinopyrum intermedium* ([Host] Barkworth & D. R. Dewey) did not reduce grain yield (Dick *et al.* 2018), perhaps because the legume seed was planted nine months later and between the Kernza drill rows set at 0.15 m spacings. The first objective of the present study was to examine the impacts on crop and pasture productivity of sowing in alternate row configurations compared to sowing species together in mixed rows to determine the effectiveness of spatial separation at sowing on reducing interspecific competition between crop and pasture species.

The transition from pasture back into crop presents different challenges. Crop production following a pasture phase in semi-arid environments is largely driven by soil water and N availability (Davies and Peoples 2003, Dear *et al.* 2004, Angus *et al.* 2006, Harris *et al.* 2008) and as a consequence, results are highly variable depending upon the climatic conditions at a particular site in a given year. Soil water availability is impacted by the prevailing seasonal rainfall, the pasture species grown (Sandral *et al.* 2006) and the timing of their removal prior to the cropping phase (Angus *et al.* 2000, Dear *et al.* 2004). Mineral N availability is also affected by environmental conditions that impact mineralisation of pasture residues, the C:N ratio of those residues and the quantum of legume biomass which is proportional to the total inputs of N returned to the soil (Angus *et al.* 2006, Peoples *et al.* 2012). Changing the row configuration of mixed pasture swards has been examined as an approach to improve the establishment, productivity and persistence of pastures sown to lucerne, subterranean clover (*Trifolium subterraneum* L.), and/or phalaris (*Phalaris aquatica* L.) (Hayes *et al.* 2017a, Hayes *et al.* 2021b). Only one study (Hayes *et al.* 2020) has examined the effects of changed row configuration of mixed pasture swards on a subsequent crop but this study, although very detailed in its sampling, only examined microplots at one site. It showed that although grain yields increased by 11% in plants grown on the original pasture drill row compared to the inter-row area there was no consistent effects of pasture type on grain yield, although wheat following phalaris-based pasture were shown to yield grain that was lower in protein compared to that following legume pastures. It is hypothesised that management strategies that improve pasture legume productivity will generally lead to a more productive transition into the cropping phase, notwithstanding the possibility in very dry years for perverse effects on year 1 crop yields due to depletion of soil water reserves from productive pastures (Latta *et*

al. 2001). In the present study, we monitor the effect of row configuration of pasture mixtures on year 1 crop performance at three contrasting sites in the Riverina region of NSW.

Methods

The transition from crop to pasture was examined at three sites, which lay in roughly an east-west transect of the Central West region of NSW, Australia. By contrast, the transition from pasture to crop was examined also on an east-west transect but in the Riverina region of southern NSW (Figure 1).



Fig. 1. Location of the experimental sites in the Central West (Cowra, Bogan Gate, Condobolin) and Riverina (Eurongilly, Wagga Wagga, Mirrool) regions of New South Wales, Australia.

Central West experiments

Three field experiments were sown on 17, 27 and 28 May 2013 at sites near Cowra (average annual rainfall 619 mm), Bogan Gate (480 mm) and Condobolin (429 mm), respectively. Experiments were repeated the following year in areas immediately adjacent to the initial experiments and sown on 6 May, 30 Apr and 1 May 2014, respectively. Details of these

experiments are described in Hayes *et al.* (2021b). Briefly, the experiments were spatially adjusted randomised split-plot designs with crop type as the main plot and row configuration as the sub-plot, with 3 replicates. Crop species included wheat (*Triticum aestivum* L. cv. Suntop) sown at 23 kg/ha, barley (*Hordeum vulgare* L. cv. Hindmarsh) at 23 kg/ha, canola (*Brassica napus* L. cv. Stingray) at 0.6 kg/ha and lupin (*Lupinus angustifolius* L. cv. Mandelup) at 40 kg/ha. All lupin seed was inoculated with Group G *Bradyrhizobium* prior to sowing using peat inoculum. The lupin treatment was excluded from experiments sown at Condobolin and Bogan Gate in 2013. Pasture species included lucerne cv. Pagasus at 3 kg/ha and subterranean clover at 3 kg/ha of each cultivar, Izmir and Seaton Park LF. Sowing rates were increased at the Cowra site on account of it being a higher rainfall environment, to 68 kg/ha for wheat and barley, 2.4 kg/ha canola, 80 kg/ha lupins, 8 kg/ha lucerne and 8 kg/ha subterranean clover cv. Coolamon. The four spatial configuration treatments included 'Pasture-only', where lucerne and subterranean clover seed were sown in every drill row in the absence of a crop; 'Crop-only' where the crop was sown in every drill row in the absence of the pasture species; 'Crop-pastureMix' where seed of lucerne, subterranean clover and the crop were sown in every drill row; and 'Crop-pasture1:1' where seed of the crop was sown separately in alternate rows to the lucerne and subterranean clover. The canola in the Condobolin experiment sown in 2014 was heavily grazed by birds during the establishment period and has been excluded from all analysis.

Plots (12 m x 4 m) were sown with a cone seeder that was fitted with two distributing cones connected to 8 tynes at a 0.25 m spacing, and with narrow points and press wheels. Plots were sown with two passes of the seeder to achieve 16 drill rows per plot. Row configuration was manipulated by placing the seed of the crop in one distributing cone and the seed of the pasture in the other, then adjusting the tubes to control the tynes to which the cones delivered seed of the respective species. Seeding rates of a particular species remained constant regardless of row configuration or the presence of companion species. That is, when seed was confined to alternate drill rows (Crop-pasture1:1), the seeding density of the drill row was doubled in order to maintain the same overall seeding rate on an area (kg/ha) basis.

Approximately six weeks after sowing, plant establishment of all species sown was determined by counting the number of seedlings in a 0.5 m length of two adjacent rows. This was carried out in two random locations of each plot, taking care to avoid edge rows. Peak crop dry matter (DM) production was assessed by cutting plants at ground level along a 0.5 m length of two adjacent rows on opposite sides and towards either end of each plot. Timing of this sampling aligned with 50% flowering for the plots containing cereals and canola and mid

pod development in plots containing lupin. The corresponding Pasture-only plot for each crop type was sampled at the same time in a similar manner. Species were separated and weighed individually in the Crop-pasture treatments

For each crop species, a 0.5 m length of four centrally located rows in each plot were cut at ground level to determine harvest index (HI). In the Crop:pasture1:1 treatment, this only involved two cropping rows. Grain was threshed using a small-scale thresher (Kimseed, Wangara, WA). The recovered grain was also used to calculate thousand kernel weight (TKW) by weighing a subsample of 100 seeds per plot. In the cereal crop treatments, the number of tillers per sample was counted and used to calculate tillers/m² of each plot. No assessment of pasture biomass was taken at this time. After sampling, the remainder of the plots were harvested with a plot harvester (Kingaroy Engineering Works, QLD) to determine final grain yield. Harvest index grain samples were ground to pass through a 2 mm screen with a bench mounted laboratory mill (Christy, Ipswich, UK) and used to determine nitrogen concentration (%) through LECO dry combustion. Protein concentration was calculated by multiplying by a factor of 5.7 for cereal crops and 6.25 for canola and lupin (Mariotti *et al.* 2008). Nitrogen content of remaining crop stubble was calculated by multiplying LECO nitrogen concentration with respective biomass values.

Pasture DM was estimated using a calibrated visual score of pasture in a 0.1 m² quadrat followed by estimation of pasture composition of the same quadrat using the dry weight rank method (Mannetje and Haydock 1963). Ten consecutive quadrats were taken on a transect perpendicular to the direction of sowing in each plot. This accounted for the vacant rows previously occupied by annual crop species in the 1:1 configuration, rather than using random quadrat placement, which may bias composition. Assessment of DM began approximately three months after grain harvest and continued at three monthly intervals until the end of the experiment.

Monthly rainfall data for the 3 sites during the experimental period are shown in Figure 2. 2013 was the driest year at Cowra (25% below the LTA) and Condobolin (19% below the LTA), but was slightly above average at the Bogan Gate site (496 mm) with 2015 being the driest year at that site, just 12% below average. By contrast, 2016 was well (>200 mm) above average in terms of total rainfall received. In general, when compared to long term rainfall readings at the respective sites, the seasonal conditions at all sites for most of the experimental period was reasonably favourable.

Riverina experiments

Three field experiments were sown to pasture in the Riverina region of southern NSW in 2012 and described in detail by Hayes *et al.* (2017a). Briefly, the experiments compared the productivity, composition and persistence of 10 pasture mixtures including lucerne, phalaris and/or subterranean clover sown in a range of row configurations. Row configurations included a 'mix' treatment, where each species were sown in every drill row, a '1:1' treatment where two species were separated and sown in alternate rows, and a '1:2' treatment where the species were again separated and the seed of the first species confined to only 1 of every 3 rows while seed of the second species was confined to the remaining two drill rows. As with the Central West experiments, seeding rate of a given species remained consistent on an 'area' (kg/ha) basis regardless of row configuration treatment.

Following termination of the pastures in 2014, experiments were retained and sown to crop in 2015. The sites were near the townships of Mirrool (A.A.R. 471 mm), Wagga Wagga (530 mm) and Eurongilly (535 mm). Whereas the latter two sites have similar rainfall, the Eurongilly site generally experiences substantially cooler temperatures, particularly during summer (Hayes *et al.* 2021b). Pastures at all sites were terminated with the application of glyphosate herbicide in mid-September 2014. Follow-up applications of herbicide were applied at the sites in December and January as required, to control newly emerged weeds as well as to kill a small number of lucerne plants that had survived the previous applications. In February/March, a small number of persistent weeds were removed by hand.

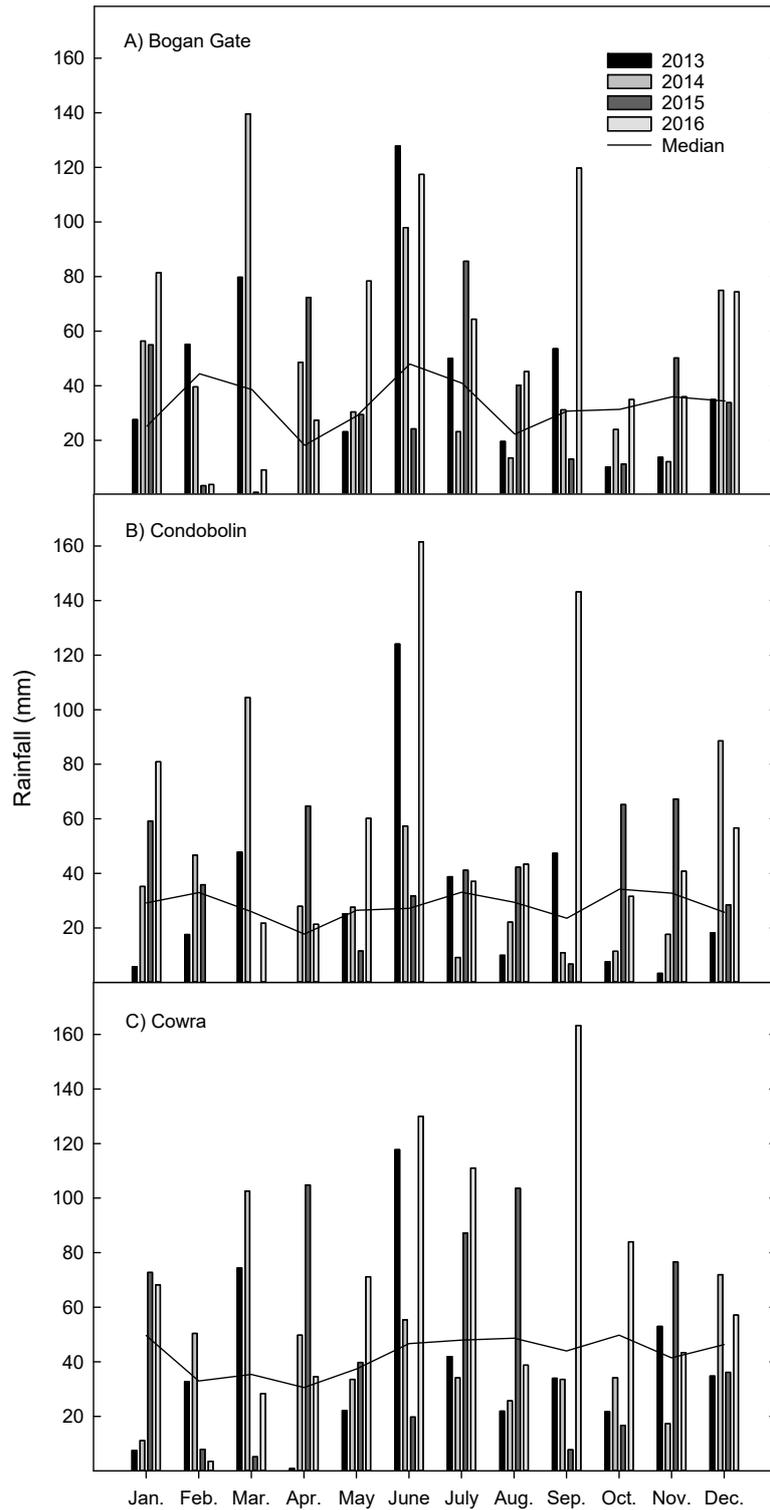


Fig. 2. Monthly rainfall (mm) recordings at the Central West sites during the experimental period, compared to the long-term median rainfall a) Bogan Gate, B) Condobolin and C) Cowra.

Soil cores were taken from the Mirrool and Eurongilly sites on 1 and 10 April 2015, respectively. Cores (42 mm in diameter) were taken to 0.6 m depth both on and between the pasture drill rows, taking care to ensure that the sampling method did not bias samples. For example, two cores taken on the drill rows were composited with two cores taken from the inter-row area per plot in the mix and 1:1 treatments. By contrast, three cores were taken both on and between drill rows, separately, from each plot sown to a 1:2 configuration, then composited, to ensure an unbiased representation of soil attributes for a given treatment. Cores were composited into the following depth increments prior to drying in a fan-forced dehydrator at 40 °C: 0-0.1 m, 0.1-0.2 m, 0.2-0.3 m, 0.3-0.4 m, and 0.4-0.6 m. Soil samples were taken at the Wagga Wagga site on 23 April using 25 mm diameter cores to 0.6 m depth. Soil samples at this site taken from specific locations within the plot (for example, from on a lucerne drill row, on a phalaris drill row or from the area between a lucerne drill row and a phalaris drill row) were sectioned into the same depth increments as described above and analysed separately. Results from that sampling formed the basis of an intensive study conducted at that one site examining spatial effects of different row configurations (Hayes *et al.* 2020). Soil data from that study have been included here for the purpose of a cross-site analysis, but plot means have been calculated with the use of weightings as required to ensure that values from a particular sampling location are appropriately represented for a given treatment.

The Mirrool and Eurongilly sites were sown on 28 and 29 April, respectively. Wheat and canola were sown over the previous pasture plots in a strip-plot design, achieved by sowing the crop perpendicular to the original pasture drill rows with three passes of a cone seeder that was 2 m in width for each crop (half the length of the original plot). Wheat (cv. Lancer) was sown at 80 kg/ha and Canola (cv. 45Y25RR) at 3 kg/ha. A mixture of single superphosphate and di-ammonium phosphate was applied at sowing such that all plots received 4.7, 7.5 and 3.3 kg/ha of nitrogen, phosphorus and sulphur, respectively. Prior to sowing, an application of knock-down herbicide, which included Roundup Ultramax (570 g/L glyphosate) at 1.9 L/ha plus Goal (240 g/L oxyfluorfen + 108 g/L N-methyl pyrrolidine) at 75 ml/ha was applied across all plots. The canola plots received an application of pre-emergent herbicide that included a mix of propyzamide 500 (500 g/kg propyzamide) at 1.1 kg/ha, Dual Gold (960 g/L S-Metolachlor) at 250 ml/ha and Trifluralin (480 g/L Trifluralin) at 2 L/ha. The pre-emergent herbicides applied to the wheat plots included Sakura (850 g/kg pyroxasulfone) at 118 g/ha and Avadex Xtra (500 g/L Tri-allate) at 2 L/ha. Canola at the Mirrool site failed to establish

evenly due to very dry seasonal conditions and so yields of canola from only the Eurongilly site are reported here.

The Wagga Wagga site was sown only to wheat cv. Gregory at 80 kg/ha on 6 May 2015, as plot size of the original pasture plot at this site (6 x 4 m) was too small to split. Single superphosphate (8.8% P, 11% S) was applied at 135 kg/ha at sowing. One week prior to sowing the site was sprayed with Roundup Max (540 g/L glyphosate) at 1.5 L/ha and Le-mat (290 g/L omethoate) insecticide at 100 ml/ha. No pre-emergent herbicide was applied at this site due to the low weed burden.

Sampling of crop biomass was as described above for the Central West experiments. No estimation of the N content of straw/chaff was made, so calculations of N content are for the grain component only. Grain from the Mirrool site was not analysed for total N concentration and kernel size was not estimated.

Seasonal conditions at the Riverina sites were characterised by a series of dry springs, including throughout the pasture phase (Hayes *et al.* 2017). Total rainfall in 2015 for the first year of crop was above average at all three sites, despite the dry September/October grain-fill period. The autumn (March-May) period at Mirrool in particular hampered crop establishment in that year (Figure 3).

Statistical analysis

Data for the Central West experiments was analysed separately to that of the Riverina experiments. For experiments in the Central West, an analysis of multiple experiments was conducted using the method of residual maximum likelihood in Genstat (VSN International 2020). The fixed effects in the model included site, year of sowing or year of sampling, crop type, row configuration, and all two-, three- and four-way interactions, and the random effects were replicates, replicate.site and replicate.year with site to specify each data unit of different experiments. For experiments in the Riverina, fixed effects included site, pasture type and the two-way interaction with replicate as a random term in the models for each analysis. Canola yields were analysed in the Riverina experiments using a one-way ANOVA as canola data was only available from the Eurongilly site.

Results

Central West experiments

Crop yields

Effects of crop type, row configuration, year of establishment, site and all two-, three- and four-way interactions on crop parameters are presented in Table 1. Due to the significant site and year of establishment effects, the data are presented at each site for each sowing year. Crops were generally higher-yielding in experiments sown in 2014 compared to 2013. Grain yields for different crops were the highest at the Cowra site and the lowest at the Condobolin site (Figure 4). Most of the 2- and 3-way interactions that included row configuration were not significant ($P>0.05$). However, there were significant Crop x Year x Row configuration and Site x Year x Row configuration interactions observed on crop establishment, which is due largely to the reductions in barley and wheat densities in the Crop-Pasture 1:1 treatments compared to other row configurations (Supplementary Figure 1).

Averaged across sites, years and crops, grain yield was reduced by 24% in 1:1 treatments (2.5 t/ha) compared to where crops were sown in every drill row in the Crop-pastureMix and Crop-only treatments (both 3.3 t/ha). The presence of pasture seedlings in year 1 in the Crop-pastureMix therefore had no impact on crop yields. Lower crop yields in the 1:1 treatments were associated with lower above-ground biomass, a lower tiller density for the cereals and, particularly at the Cowra site in 2013, a lower establishment density (Supplementary Figures 1 and 2). There was no advantage in terms of grain size or harvest index associated with the reduced yield, with values for both parameters being lower in the 1:1 treatment compared to

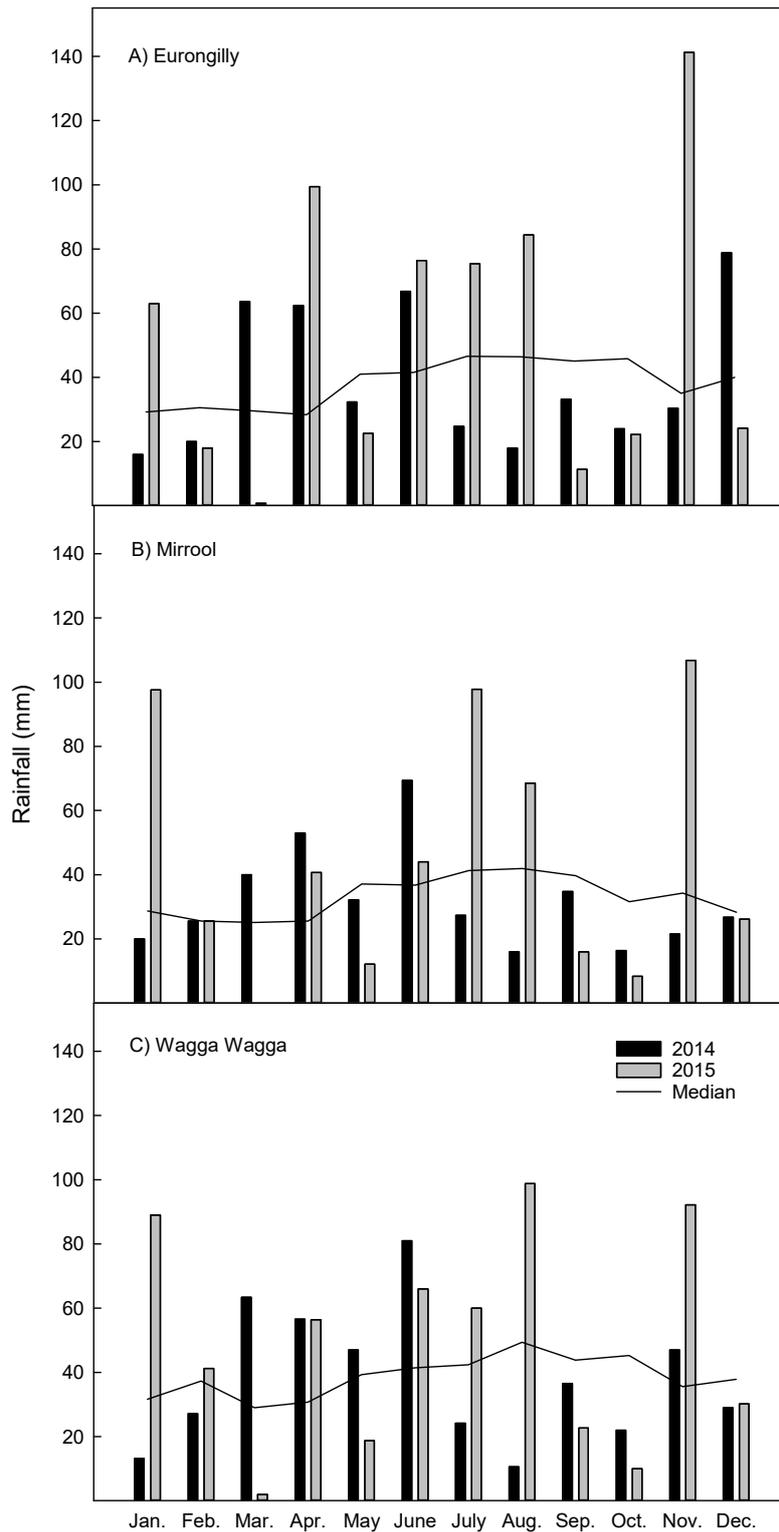


Fig. 3. Monthly rainfall recordings at A) Eurongilly, B) Mirrool and C) Wagga Wagga in the final year of pasture (2014) and the first year of the crop rotation (2015).

Table 1. Analysis of multiple experiments using the method of residual maximum likelihood for crop parameters at the Central West sites.

Strata/Decomposition	Effect ^A	Establishment count	Tiller density	Anthesis DM	Harvest DM	Grain yield	Harvest index	TKW	Grain N	Straw&Chaff N	Total N content	%Grain N
Experimental units												
Crop	F	167.94***	631.07***	60.83***	73.86***	269.19***	1540.47***	11361.30***	9303.05***	242.07***	47.53***	373.95***
Row configuration	F	3.72*	24.33**	34.50***	28.56***	28.80***	0.04n.s.	3.41*	4.83**	0.48n.s.	22.12***	0.31n.s.
Crop × Row configuration	F	1.01n.s.	2.04n.s.	1.45n.s.	0.82n.s.	2.03n.s.	1.07n.s.	2.74*	2.88*	0.91n.s.	1.48n.s.	0.57n.s.
Residual	R											
Experimental units × Year ^B												
Year	F	8.21n.s.	55.17**	11.60**	35.52**	14.50**	14.05***	560.84***	3727.20***	1276.03***	399.91***	154.58***
Year × Crop	F	0.85n.s.	69.50***	61.58***	17.92***	23.83***	6.59***	7.93***	12.55***	4.46**	27.81***	14.61***
Year × Row configuration	F	0.51n.s.	1.50n.s.	0.73n.s.	0.10n.s.	0.08n.s.	0.95n.s.	1.84n.s.	0.17n.s.	1.02n.s.	2.36n.s.	0.24n.s.
Year × Crop × Row configuration	F	2.40*	1.68n.s.	1.62n.s.	0.37n.s.	0.30n.s.	0.32n.s.	0.82n.s.	1.12n.s.	0.44n.s.	0.42n.s.	0.55n.s.
Residual	R											
Experimental units × Site × Year												
Site	F	280.26***	109.45***	181.24***	298.05***	205.26***	300.66***	332.64***	241.08***	54.42***	317.01***	318.95***
Site × Crop	F	22.96***	8.63***	5.58***	19.31***	17.06***	202.00***	102.51***	98.36***	48.79***	6.24***	236.23***
Site × Row configuration	F	3.12*	5.13**	1.47n.s.	5.80***	4.98***	2.30n.s.	1.01n.s.	0.39n.s.	0.29n.s.	4.32**	0.81n.s.
Site × Crop × Row configuration	F	2.10*	0.52n.s.	0.97n.s.	1.08n.s.	0.96n.s.	0.88n.s.	1.10n.s.	1.12n.s.	1.80n.s.	0.68n.s.	1.97*
Site × Year	F	1.01n.s.	11.56***	17.36***	21.75***	35.99***	94.34***	7.34***	15.14***	8.54***	50.23***	85.02***
Site × Year × Crop	F	0.05n.s.	4.30*	7.32***	3.63*	1.99n.s.	0.38n.s.	1.01n.s.	4.82**	0.90n.s.	1.68n.s.	3.47*
Site × Year × Row configuration	F	2.48*	2.41n.s.	1.55n.s.	0.55n.s.	0.67n.s.	0.63n.s.	0.90n.s.	0.76n.s.	1.11n.s.	1.21n.s.	0.80n.s.
Site × Year × Crop × Row configuration	F	1.29n.s.	0.32n.s.	0.82n.s.	0.30n.s.	0.80n.s.	1.62n.s.	0.23n.s.	0.63n.s.	0.59n.s.	0.37n.s.	1.60n.s.
Residual	R											

^AF, Fixed effect; R, Random effect; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significant. ^BCrop year of establishment.

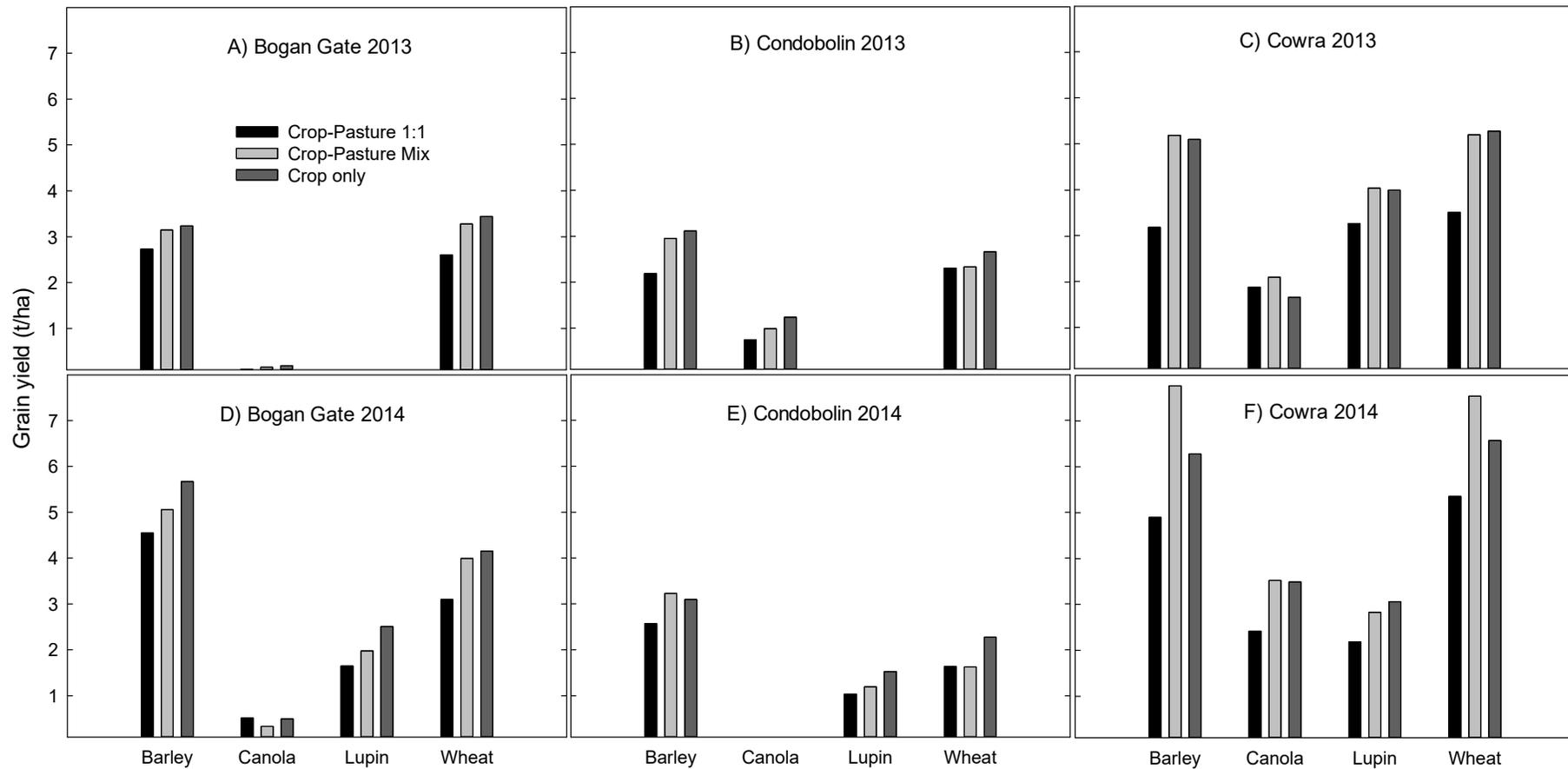


Fig. 4. Grain yield (t/ha) of barley, canola, lupin and wheat sown in different spatial configurations at Bogan Gate (A & D), Condobolin (B & E) and Cowra (C & F) in 2013 (A, B & C) and 2014 (D, E & F).

the Crop-pastureMix and Crop-only treatments, although differences in harvest index were not significant ($P>0.05$). The total N content of above-ground crop biomass (stubble plus grain) was lower ($P<0.05$) in the Crop-pasture1:1 treatment (average 86 kg N/ha) compared to the Crop-pastureMix (110 kg N/ha) and Crop-only treatments (112 kg N/ha). Crop accumulation of N was highest ($P<0.001$) in lupin (149 kg N/ha) and lowest in canola (78 kg N/ha), which was not significantly different to wheat (96 kg N/ha) or barley (98 kg N/ha). Grain N content represented 75% of total N content of all crops except canola, where grain N was a significantly lower portion of total N, at only 53% ($P<0.05$).

Year one pasture DM

The effects of row configuration, crop type, year of establishment, site and all two-, three- and four-way interactions on year 1 pasture dry matter (DM) at anthesis (of the respective crop) is presented in Table 2. Averaged across experiments, year 1 subterranean clover DM was three-fold greater in the pasture-only treatments (1.8 t/ha) compared to where a cover crop was sown (average 0.6 t/ha), and was ~70% greater where it was separated from the crop in alternate rows (0.8 t/ha, or 11% of total DM) compared to where all species were mixed in the same drill row (0.5 t/ha, or 6% of total DM). Subterranean clover and lucerne comprised 35% and 43% of total DM in the pasture-only treatment in spring year 1, with unsown weeds making up the difference. By comparison, lucerne comprised only 15% and 19% of total DM in the mixed and 1:1 configurations, respectively. Averaged across sites, years and row configuration treatments, barley (63%) wheat (67%), lupins (55%) and canola (45%) comprised the largest single component of spring biomass in year 1, with pastures and unsown weeds making up the balance. Averaged across all experiments, average total year 1 legume (lucerne plus subterranean clover) DM in the pasture-only treatment was 4.1 t/ha. By comparison, legume DM was only 2.2 t/ha in the Crop-pasture1:1 treatment and 1.6 t/ha in the mixed drill row treatment where there was a corresponding increase in crop biomass due to the alternate row configuration. For example, barley biomass increased from 4.5 t/ha in the Crop-pasture1:1 treatment to 5.5 t/ha in the Crop-pastureMix treatment. Similarly, wheat yields increased from 5.5 t/ha to 7.5 t/ha and lupin yields increased from 5.1 t/ha to 5.9 t/ha. Canola was the exception with little difference in crop biomass between the mix (3.0 t/ha) and 1:1 (3.1 t/ha) treatments. Averaged across row configuration treatments at experiments where all crop species were tested, total pasture biomass was highest under a lupin cover crop (3.5 t/ha), compared with pasture under canola (3.1 t/ha), wheat (2.7 t/ha) and barley (2.5 t/ha).

Table 2. Analysis of multiple experiments using the method of residual maximum likelihood for cumulative pasture DM production at the Central West sites

Strata/Decomposition	Effect ^A	Sites sown 2013				Sites sown 2014			
		Total DM	Lucerne	Subclover	Weeds	Total DM	Lucerne	Subclover	Weeds
Experimental units									
Crop ^B	F	24.08***	10.51***	12.76***	6.37***	22.95***	5.77***	8.32***	1.71n.s.
Row configuration	F	70.04***	262.06***	93.77***	23.73***	54.56***	212.11***	85.12***	11.69***
Crop × Row configuration	F	6.38***	3.13**	4.08**	0.87n.s.	2.47*	2.25*	1.58n.s.	2.13*
Residual	R								
Experimental units × Year ^C									
Year	F	514.29***	293.12***	76.87***	63.68***	549.03***	17.48***	95.95***	45.72***
Year × Crop	F	20.87***	8.22***	11.69***	13.12***	26.13***	1.01n.s.	4.45**	1.92n.s.
Year × Row configuration	F	63.99***	96.66***	64.79***	41.41***	131.80***	12.09***	30.16***	17.19***
Year × Crop × Row configuration	F	3.54***	2.27**	2.27**	0.81n.s.	5.28***	0.75n.s.	1.13n.s.	1.12n.s.
Residual	R								
Experimental units × Site × Year									
Site	F	1187.93***	726.20***	487.10***	9.08*	1096.08***	795.26***	250.77***	10.14*
Site × Crop	F	1.94n.s.	1.22n.s.	1.67n.s.	7.40***	5.06***	12.27***	3.17**	1.69n.s.
Site × Row configuration	F	85.94***	99.95***	64.44***	11.80***	96.49***	114.11***	41.52***	6.40***
Site × Crop × Row configuration	F	1.88*	3.36***	0.96n.s.	0.89n.s.	4.61***	9.49***	1.82*	1.91*
Site × Year	F	203.71***	153.02***	31.42***	80.38***	430.32***	545.71***	102.74***	6.62**
Site × Year × Crop	F	1.27n.s.	0.87n.s.	1.77n.s.	5.30***	10.44***	6.63***	2.62*	3.53**
Site × Year × Row configuration	F	31.49***	26.98***	15.42***	24.15***	68.18***	91.03***	22.37***	7.31***
Site × Year × Crop × Row configuration	F	0.73n.s.	1.14n.s.	0.82n.s.	0.92n.s.	4.14***	5.66***	0.47n.s.	1.64n.s.
Residual	R								

^A F, Fixed effect; R, Random effect; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significant. ^B Prior crop. ^C Pasture year (Pastures sown in 2013 for 3 years, pastures sown in 2014 for 2 years)

Pasture DM post-establishment year

Due to significant site, prior crops and pasture growing year effects, the data were presented at each site at each year under different crop types. Cumulative total pasture production in all experiments over the experimental period was 5 times greater at the Cowra site than at Condobolin or Bogan Gate (Figures 5, 6 and 7).

In both Cowra experiments, there was no consistent yield advantage beyond the establishment year in the Pasture-only treatments compared to pastures sown with a cover crop in mixed drill rows. However, pastures sown to the 1:1 configuration yielded 17-29% less than the Crop-pastureMix treatment in years 2 and 3, respectively. Subterranean clover composition was consistently higher in the 1:1 treatment over the life of the experiments, comprising 27% and 35% of total DM in years 2 and 3 respectively, compared to 13% and 11% in the Crop-pastureMix and 15% and 13% in the Pasture-only treatments, respectively (Figure 7). By contrast, lucerne composition remained lower in the Crop-pasture1:1 (average 52% of total DM) compared to the Crop-Pasturemix swards (78%), which were similar to levels observed in the Pasture-only sward (81%). Although weed incursions in all treatments sown to pasture were generally very low, the composition of weeds was often greater in the Crop-Pasture1:1 (average 16% of total DM) compared to the Crop-pastureMix (9%) and Pasture-only (5%) treatments. Weed composition was always greatest, and total biomass least, in the Crop-only treatments.

Trends were similar at the Bogan Gate and Condobolin experiments, although pasture yields were much less than at the Cowra site. Total DM in the Crop-Pasture1:1 treatment was on average 16% lower compared to the Crop-pastureMix configuration, except for year 3 at Condobolin where yields were higher in the 1:1 (average 1.0 t/ha) compared to the mix treatments (0.8 t/ha). Subterranean clover declined to negligible levels at both sites (<500 kg/ha annual biomass) beyond year 1, even where it was sown in the absence of a cover crop. The level of weed incursion was consistently greater throughout the pasture phase in the mix (average across all years at both sites of 22% of total DM) and 1:1 (33%) treatments, compared to where pasture was established without a cover crop (16%) in year 1. Averaged across row configuration treatments at experiments where all crop species were tested (including the Cowra sites), yearly pasture biomass was broadly similar following lupin (average 12.0 t/ha), canola (11.8 t/ha), wheat (10.5 t/ha) and barley (10.9 t/ha) cover crops.

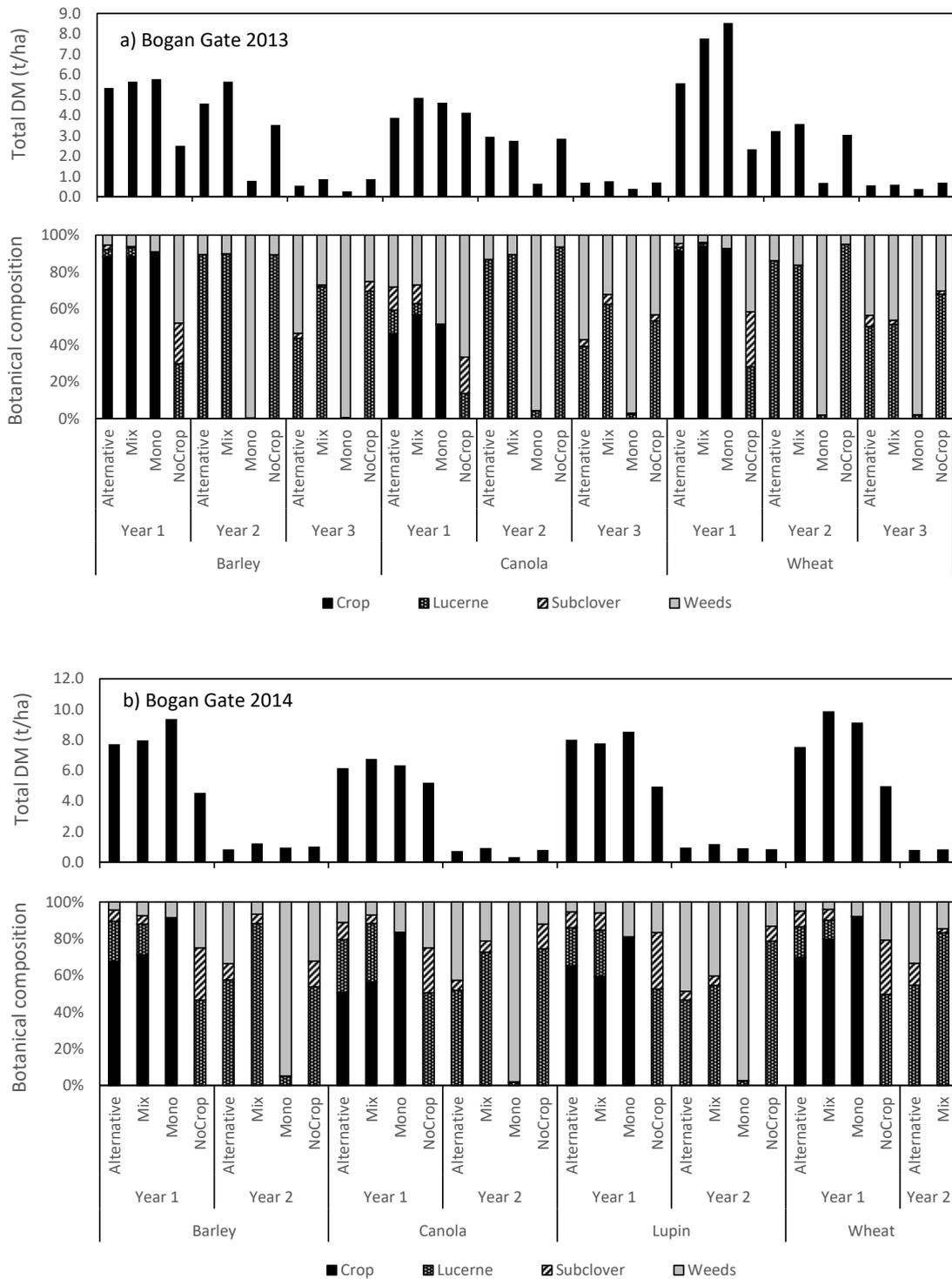


Fig. 5. Aboveground biomass (t/ha) and botanical composition (%) of swards sown at Bogan Gate in a) 2013 or b) 2014, with or without barley, canola, lupin or wheat cover crops in alternate (1:1) or mixed row configurations.

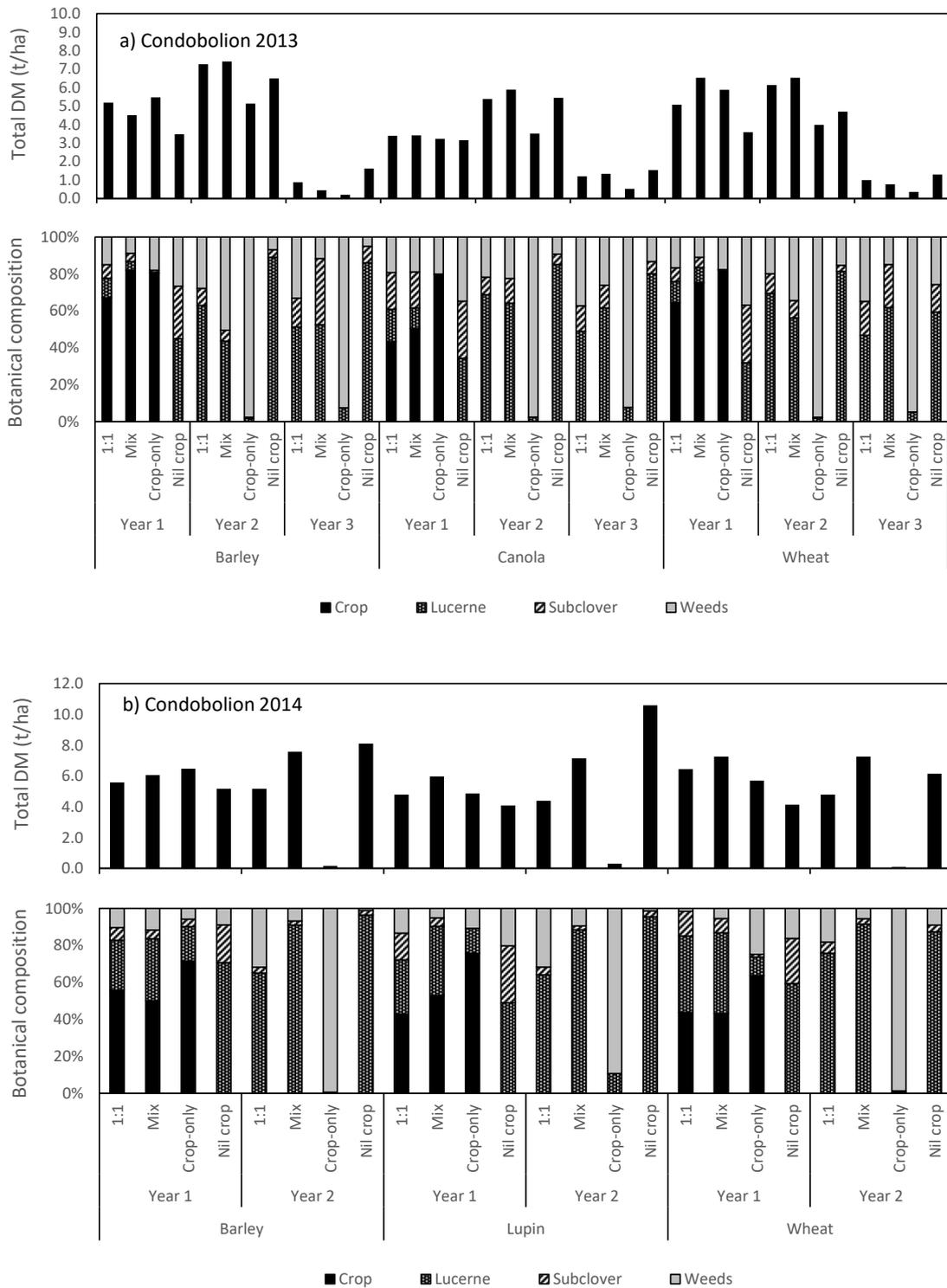


Fig. 6. Aboveground biomass (t/ha) and botanical composition (%) of swards sown at Condobolin in a) 2013 or b) 2014, with or without barley, canola, lupin or wheat cover crops in alternate (1:1) or mixed row configurations.

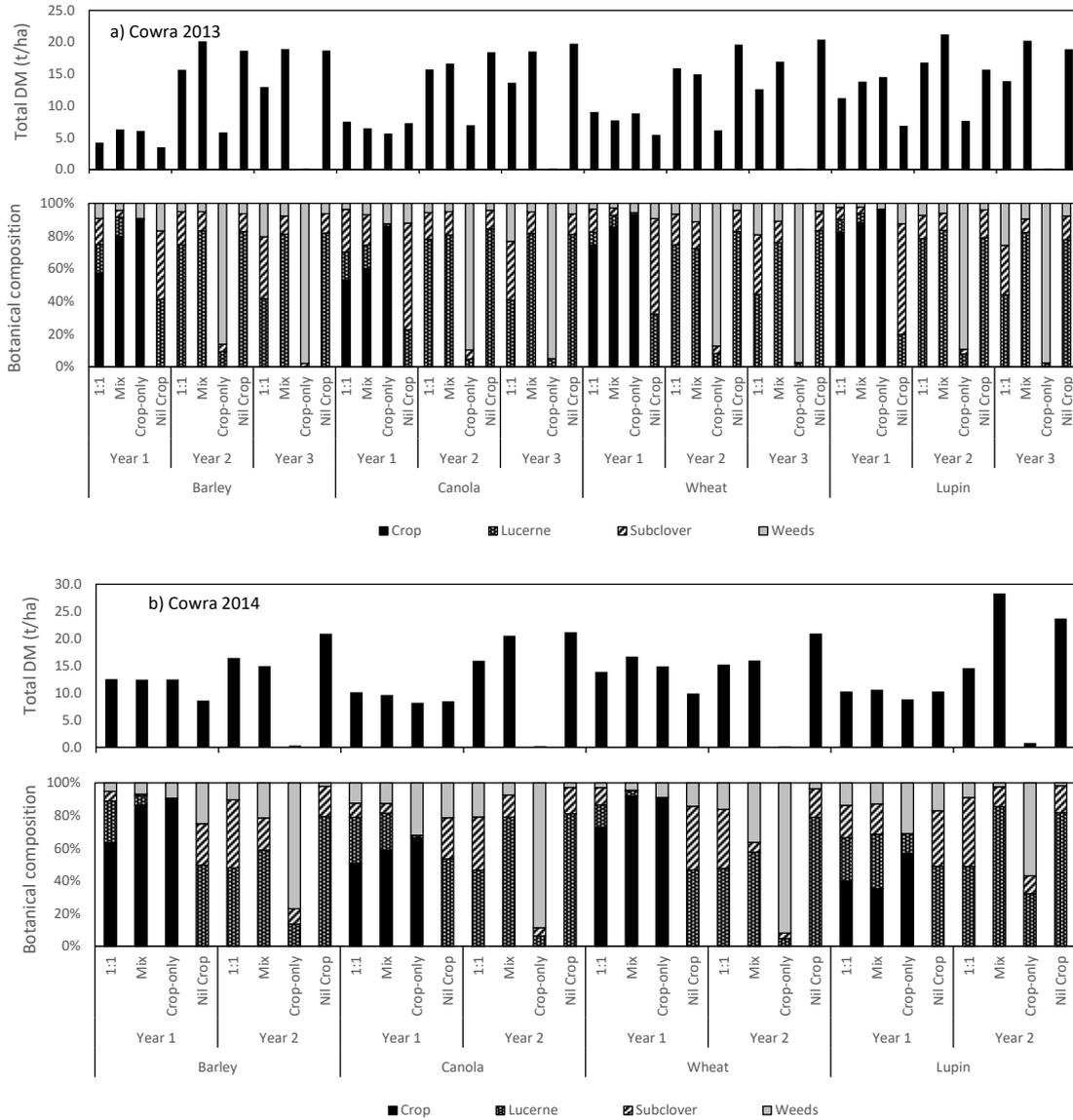


Fig. 7. Aboveground biomass (t/ha) and botanical composition (%) of swards sown at Cowra in a) 2013 or b) 2014, with or without barley, canola, lupin or wheat cover crops in alternate (1:1) or mixed row configurations.

Riverina experiments

Soil mineral N

There was a significant ($P < 0.05$) site x pasture treatment interaction (Figure 8) in the quantum of mineral N detected in the 0-0.6 m soil depth following the removal of pastures, just prior to sowing the crops. There was more mineral N at the Eurongilly site (average 266 kg/ha) compared to either Mirrool (157 kg/ha) or Wagga Wagga (150 kg/ha). Averaged across sites, mineral N was greatest in the Lucerne-only plots (240 kg/ha) which was significantly higher than in the Phalaris/subterranean clover mix (176 kg/ha), Phalaris/lucerne mix (160 kg/ha), Phalaris/lucerne 1:1 (158 kg/ha) and Phalaris/subterranean clover 1:1 (142 kg/ha) treatments. At the Wagga Wagga site, mineral N was greatest in the subterranean clover-only treatment but at Mirrool this treatment had amongst the lowest mineral N values of all treatments.

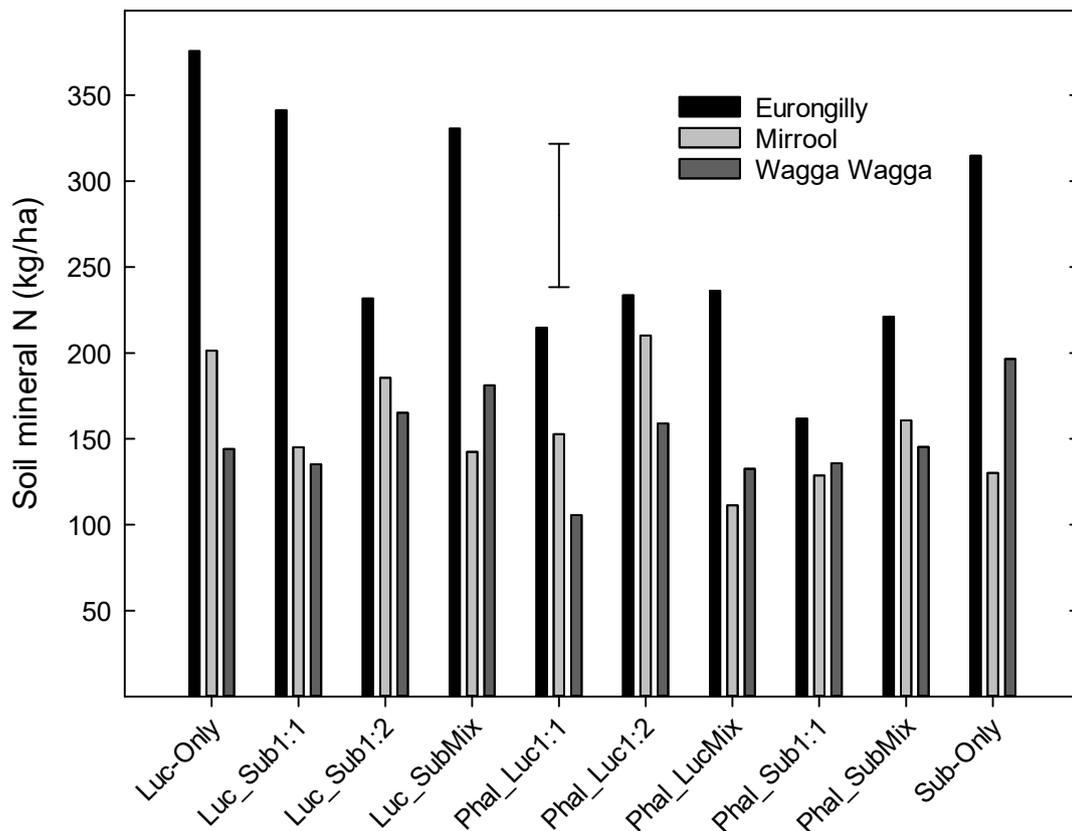


Fig. 8. Soil mineral N (kg/ha) in the 0-0.60 m soil profile at Eurongilly, Mirrool and Wagga Wagga following different pasture combinations and configurations. Error bar indicates l.s.d. for the significant site x pasture type interaction at $P=0.05$.

Crop yields and grain attributes

There was no significant site x pasture type interaction on wheat yields or grain attributes, thus site effects and pasture treatment effects are presented separately (Tables 3 and 4). Grain protein was lowest in wheat following phalaris/subterranean clover swards, highest in lucerne/subterranean clover swards and inversely related to kernel size ($P < 0.001$, $r^2 = 0.79$, $n=36$; $y = -1.836x + 48.56$). Tiller density tended to be lower following swards containing phalaris compared to lucerne/annual legume mixtures (Table 4). Total N content of grain was significantly higher at Eurongilly compared to the Wagga Wagga site, reflecting greater yields and a higher protein concentration of grain from the Eurongilly site (Figure 9). At both sites, grain N content was highest in the Lucerne/subterranean clover 1:1 and 1:2 treatments and lowest in the Phalaris/subterranean clover treatments. However, the total N content of grain following the pure lucerne treatment was not significantly different to the Phalaris/subterranean clover treatments at the Wagga Wagga site which was in contrast to the response observed at the Eurongilly site where grain N content following the lucerne-only treatment was among the highest of any treatment.

Table 3. Significant main effects of site on wheat establishment density (plants/m²), biomass (DM) at harvest (t/ha), grain yield (t/ha) and harvest index.

Site	Seedling density	Crop DM	Grain yield	Harvest index
Eurongilly	149	14.8	6.2	41.9
Mirrool	94	11.0	4.3	39.0
Wagga Wagga	276	13.7	4.8	35.4
<i>l.s.d.</i> _{0.05}	8.2	0.37	0.18	0.99

Table 4. Main effects of prior pasture treatment on wheat establishment count (plants/m²), tiller density (per m²), anthesis and harvest DM (t/ha), thousand kernel weight (TKW; g) grain nitrogen concentration (N; %) and grain protein (%) averaged across three Riverina sites at Eurongilly, Mirrool and Wagga Wagga, NSW.

Treatment	Establishment count	Tiller density	Anthesis DM	Harvest DM	Grain yield	Harvest index	TKW	Grain protein
Lucerne_Only	167	414	12.0	13.0	5.2	39.4	31.3	12.5
Lucerne_Subclover_1:1	179	482	12.4	13.2	5.1	38.1	27.5	13.3
Lucerne_Subclover_1:2	174	499	12.9	13.3	5.2	38.7	27.3	13.7
Lucerne_Subclover_Mix	169	440	12.6	13.0	4.9	37.8	27.3	13.6
Phalaris_Lucerne_1:1_Subclover	175	430	12.4	13.3	5.2	38.9	29.1	12.2
Phalaris_Lucerne_1:2_Subclover	178	431	12.4	13.4	5.2	38.7	28.5	12.5
Phalaris_Lucerne_Mix_Subclover	175	377	12.0	12.9	5.1	39.4	30.0	12.7
Phalaris_Subclover_1:1	168	374	11.9	13.2	5.2	39.4	33.5	10.0
Phalaris_Subclover_Mix	175	377	11.4	12.6	4.8	38.4	32.3	10.6
Subclover_Only	174	434	12.7	13.5	5.3	39.0	27.6	12.9
Significance	ns	***	ns	ns	ns	ns	***	***
<i>l.s.d.</i> _{0.05}	-	40.4	-	-	-	-	1.37	0.71

ns, differences not significant at P = 0.05; ***, P < 0.001.

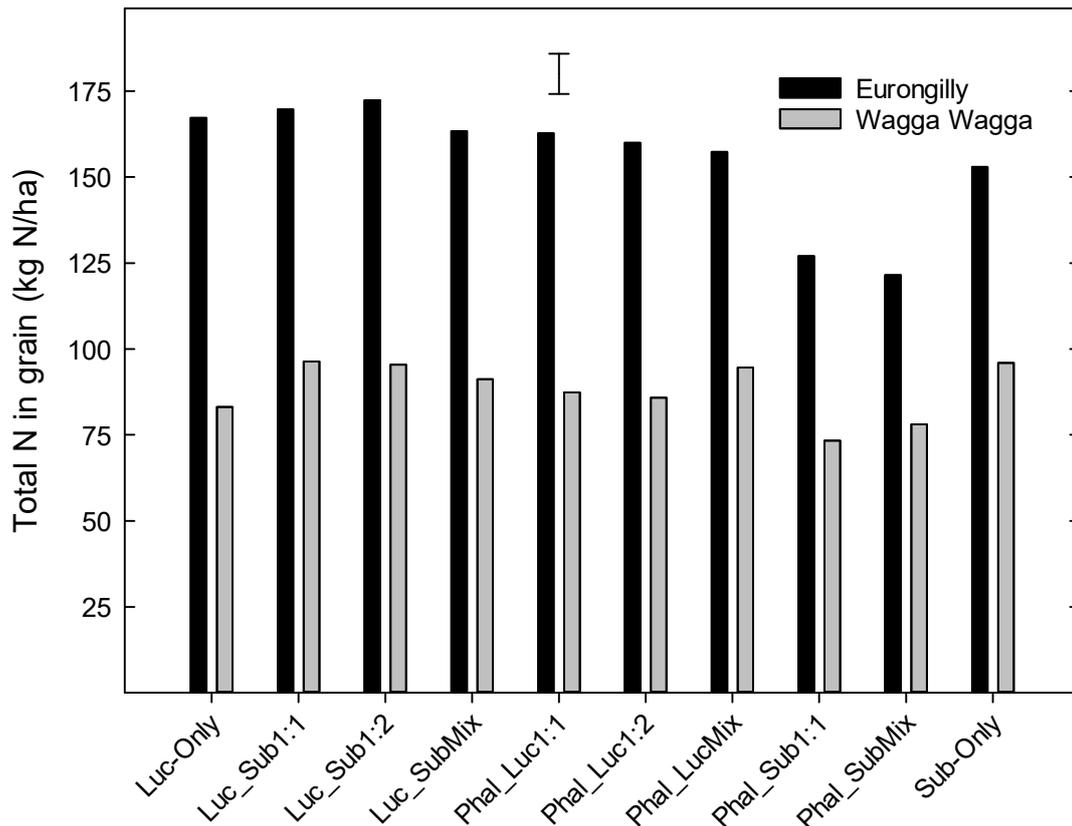


Fig. 9. Total nitrogen (N) content of grain harvested from wheat following different pasture combinations and configurations at Eurongilly and Wagga Wagga. Error bar indicates l.s.d. for the significant site x pasture type interaction at $P=0.05$.

Canola yields at the Eurongilly site were largely unaffected by pasture treatment in the previous year. Crop DM at harvest was lowest ($P<0.05$) following the phalaris/subterranean clover swards (10.3 t/ha) but this did not translate into differences in yield (average 3.6 t/ha) or harvest index (average 31.0). There was also no difference in canola density due to previous pasture treatment, with an average plant density of 51 plants/m².

Discussion

Transition from cropping to pasture phase

This study examined the effects of cover crop species and row configuration at sowing to determine optimal strategies for transitioning from a cropping to a pasture phase across a range of non-irrigated, semi-arid environments in south-eastern Australia. The presence of pasture seedlings in the Crop-pastureMix treatment had no impact on grain yields of the cover-crops compared to the Crop-only treatment, notwithstanding some particular site/year effects, indicating crops were scarcely affected by intraspecific competition with pasture

species. This undoubtedly reflected the slower growth rates of lucerne and subterranean clover compared to crop seedlings. However, crop yields were depressed by an average of 24% in the 1:1 treatment compared to the Crop-pastureMix plots where the crop was sown in every drill row. Reduced crop yields are common with increased row spacing (Scott *et al.* 2013) and the row spacing of all species in the Crop-pasture1:1 treatment was effectively doubled from 0.25 m to 0.50 m. Reduced yields are generally not explained by differences in plant density, as seeding rates were consistent for each species at particular sites, although there was evidence of reduced cereal density at the Cowra site in the Crop-pasture1:1 treatment compared to other row configurations. Rather, the result is more a reflection of reduced biomass in the Crop-pasture1:1 treatment, which in the cereals was associated with reduced tiller numbers. The result is suggestive of reduced access of crops to resources in the alternate row configuration, most likely light, water, space and/or plant-available soil N.

There was little in our data to suggest that direct competition for N contributed to reduced biomass; yield of the N-fixing lupin was as much affected as any other crop, there was no discernible increase in grain protein attributable to row configuration, and as a result of depressed yields, N-demand (N-content) was lower in the Crop-pasture1:1 treatment compared to other row configurations. At the one experiment (Cowra 2013), where soil water was monitored using a neutron moisture meter, water content of the bulk soil in the Crop-pasture1:1 treatment was similar to other treatments in the establishment year (Hayes *et al.* 2021b). Similarly, the point-in-time assessment of light interception of plots in some of these experiments which was conducted in early spring also failed to distinguish effects of row configuration on total light interception (Hayes *et al.* 2021b). Instead, results more likely reflect subtle and transient restrictions in access to resources imposed by the alternate row configuration which would require more intensive sampling to detect. The concentration of the same number of plants in fewer drill rows mean that plant roots and shoots need to travel further to access all available resources. For below-ground resources, it takes time for roots to explore soil in the inter-row area. However, plants with an erect growth habit are limited in their ability to achieve full canopy cover due to their fixed location and increased intraspecific competition at a small spatial scale, associated with the concentration of seeds in fewer drill rows (Harper 1977). The increased time required to fully explore the soil volume, combined with impaired ability to achieve early canopy cover (Donald 1963) likely explain reduced crop yields associated with wider row spacing.

Subterranean clover was more responsive to the alternate row configuration than lucerne. There are several contributing factors to this result. Firstly, lucerne is more tolerant to

competition from a cover crop in the establishment year than other pasture species due largely to its summer-growth habit, enabling it to develop after the cover crop has senesced (Swan *et al.* 2014). By contrast for persistence, the self-regenerating subterranean clover needs to set seed at the same time that the cover crops are filling grain. Due to this temporal difference in physiological development, it might be said that lucerne is in less direct competition with the winter-growing annual crops than subterranean clover. Secondly, subterranean clover is known to be inherently sensitive to shading (Black 1963, Dear *et al.* 1998), making it particularly vulnerable to competition from vigorous crops (Santhirasegaram and Black 1967). Thirdly, the prostrate growth habit of subterranean clover provides increased capacity for shoots to grow laterally into the inter-row area, albeit still unlikely to fully utilise that space in the short-term in most semi-arid environments (Hayes *et al.* 2021b).

Despite the positive response to alternate row configuration observed in subterranean clover, total pasture yield did not increase. Indeed, pasture yields post-establishment year were generally lower in the Crop-pasture1:1 treatment compared to the Crop-pastureMix or Pasture-only treatments. Similar to the observations of Scott *et al.* (2013), yield differences were less evident under less favourable growing conditions. In part, the lack of yield response reflects the relatively low composition of subterranean clover in most of the pasture swards. This may be due to the fact that subterranean clover was always in mixed rows with lucerne in all treatments, which in semi-arid environments is known to be a fierce competitor with subterranean clover, especially under dry seasonal conditions (Dear and Cocks 1997, Hayes *et al.* 2017a). Also, lower pasture yields can be attributed to the wider spacing of lucerne rows in the alternate row configuration. Beyond the establishment year, every second drill row was effectively blank, covered only with the residual stubble of the crop from year 1. Lucerne density was similar regardless of row configuration treatment and remained concentrated in the drill rows into which it was originally sown (Hayes *et al.* 2021b). As lucerne was the dominant component of most swards beyond year one, total sward yields very much reflected total lucerne yields, which were likely constrained in their ability to utilise light compared to swards where lucerne was at 0.25 m row spacings.

Pasture production was lower under a cereal compared to canola or lupin cover crop, undoubtedly reflecting the smaller accumulation of biomass by the latter two crops. In the case of the pastures following lupins, Hayes *et al.* (2021b) reported reduced lucerne mortality compared to all other cover crop species, although this was more evident at the Bogan Gate and Cowra 2014 sites than at Condobolin and Cowra in 2013. The body of previous research that has examined the use of cover crops for the establishment of pastures has focused

overwhelmingly on the use of cereal cover crops (McCormick *et al.* 2014), with very few examining lupin (Hoen and Oram 1967) or brassica species (Waddington and Bittman 1984). Use of these broadleaf alternatives to cereals would appear to offer promise for the establishment of pastures in semi-arid environments such as south-eastern Australia, at least from a pasture production perspective, and could simplify the management of in-crop herbicides, where legumes are the dominant pasture species being sown. Other traits that may benefit pasture production, beyond reduced interspecific competition associated with yield, include the small seed size of the brassicas, which is similar to pasture legumes and may help the farmer set a uniform sowing depth for all species; or the N-fixing attributes of lupins, which may reduce early competition for N, particularly when establishing non-legume pasture species that are traditionally difficult to establish under a cereal cover crop (Li *et al.* 2014, Swan *et al.* 2014).

Transition from pasture to cropping phase

Effects on grain yield components could only be attributed to row configuration of the previous pasture to the extent that row configuration changed pasture composition. Consistent with previous studies in similar environments (Dear *et al.* 2004, Dear *et al.* 2009), grain size was larger and protein content lower in cereals following grass-based pastures, attributable to lower levels of mineral N in the soil prior to the start of the cropping phase. The quantum of N inputs to soil during the pasture phase is closely linked to legume biomass (Peoples *et al.* 2012), which is commonly reduced in these environments where there is a strong grass component (Hayes *et al.* 2018). However, there was no effect of previous pasture treatment on grain yield.

Subterranean clover benefitted more at establishment than lucerne from spatial separation from the cover crop. At the Riverina sites, treatments that spatially separated subterranean clover from the perennial species were also shown to enhance subterranean clover composition throughout the pasture phase (Hayes *et al.* 2017a). This network of field sites demonstrated that the relative importance of subterranean clover to total legume biomass was site dependant, and nowhere was subterranean clover more important than at the Wagga Wagga site, in a soil where lucerne growth was impaired, apparently by an aeolian layer in the profile at around 1.0 m depth that constrained root penetration and water extraction (Hayes *et al.* 2010). By contrast, subterranean clover was at a relative disadvantage to lucerne at the drier Mirrool site, where seasonal conditions did not favour seed set and regeneration of the annual species (Hayes *et al.* 2017a, Hayes *et al.* 2021b). At the Eurongilly

site, neither species appeared to be especially constrained, hence the greater soil mineral N across the pasture treatments.

Concluding remarks

Taken together, these findings suggest that in order to alter row configuration for optimal results, an understanding of the dominance hierarchy of species will be required, which will likely differ between sites. Ultimately, the aim of changed drill row configuration in mixed swards is to manage interspecific competition between sward components, particularly at the seedling stage (Harper 1977). Sasaki and Lauenroth (2011) contend that sward productivity is regulated over time by the presence of dominant species, rather than by diversity *per se*. In swards that contain a mix of annual and perennial species, the dominance hierarchy is further complicated by the time of emergence of individual seedlings relative to their neighbours (Ross and Harper 1972), highlighting the contrast in competition dynamics in the establishment year compared with subsequent years in a pasture phase. By definition, when one changes row configuration as we have done in these series of experiments, the aim is to artificially manage the dominance hierarchy through modifying the 'space' available to individual plants, as well as the distance from and identity of their neighbours. In doing so, we risk constraining the productivity of the dominant species in order that other species can co-exist and in a production context, the risk of perverse outcomes is obvious. In the present study, the establishment of lucerne in alternate rows with a cover crop served merely to reduce pasture productivity post-establishment year.

That said, the practice of altering spatial configurations in the establishment of a pasture to improve productivity in phased cropping rotations offers promise and so should not be disregarded out of hand. The greatest potential in semi-arid environments was in enhancing the establishment and productivity of the self-regenerating annual legume, subterranean clover, among competitive perennial species. Opportunities exist to mitigate the risk of perverse outcomes by minimising the distance between drill rows. The method used by Dick *et al.* (2018) of planting the legume 'between' grass or crop drill rows would seem to be preferable to our approach of concentrating seed in fewer drill rows, as the reduction in productivity of the dominant species is much less likely. The approach of Dick *et al.* (2018) which serves to reduce the effective row spacing of the sward has many benefits to production systems (Scott *et al.* 2013). This approach would also achieve greater plant cover across the landscape, which is likely to enhance soil fertility at the soil surface (Hayes *et al.* 2020) and foster a more diverse microbiome compared to wider row spacings (Hayes *et al.*

2021a). The ability for farmers to implement such narrow plantings in a commercial context, and the reliability of such an approach in achieving more diverse swards without perverse outcomes over a range of environments remain topics for further research.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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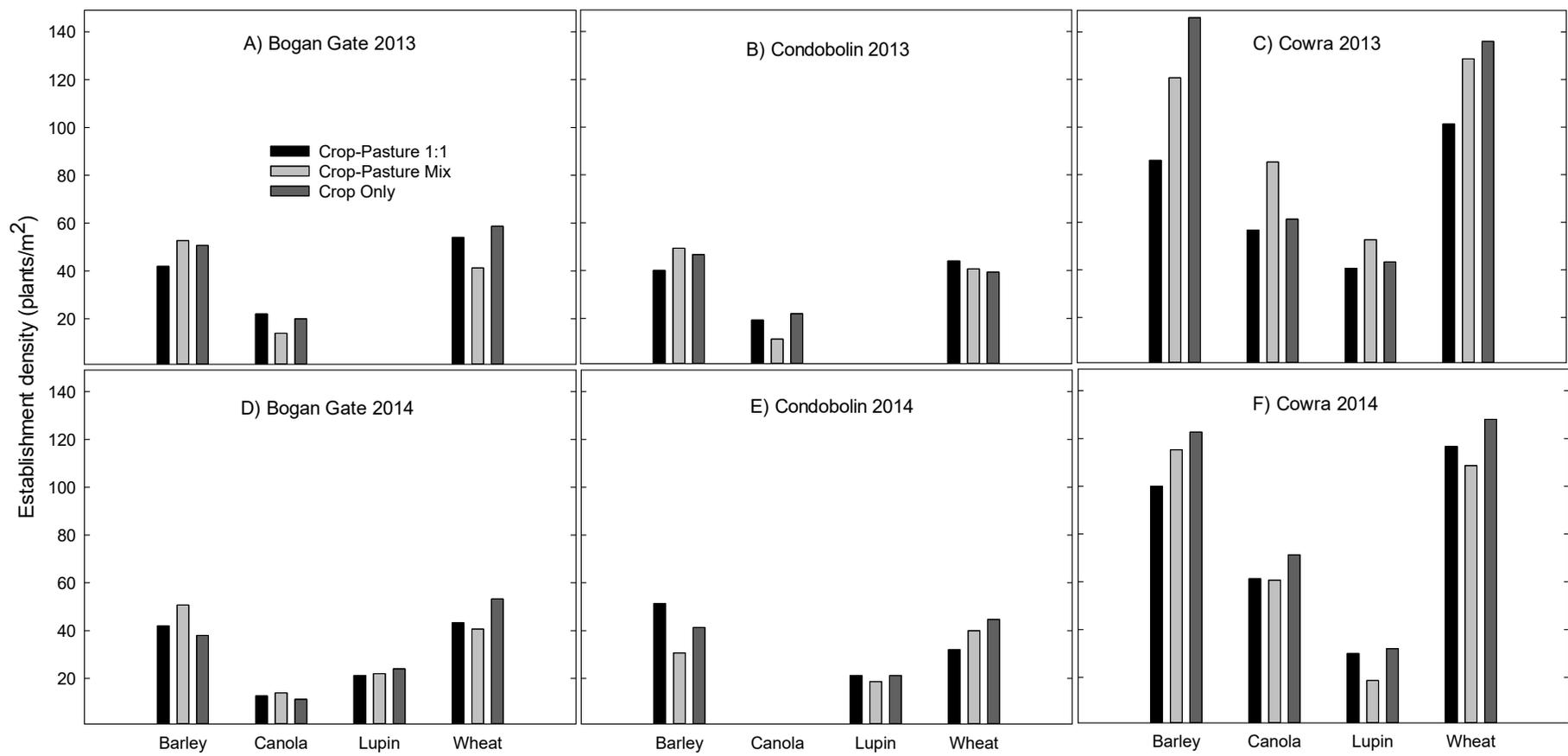
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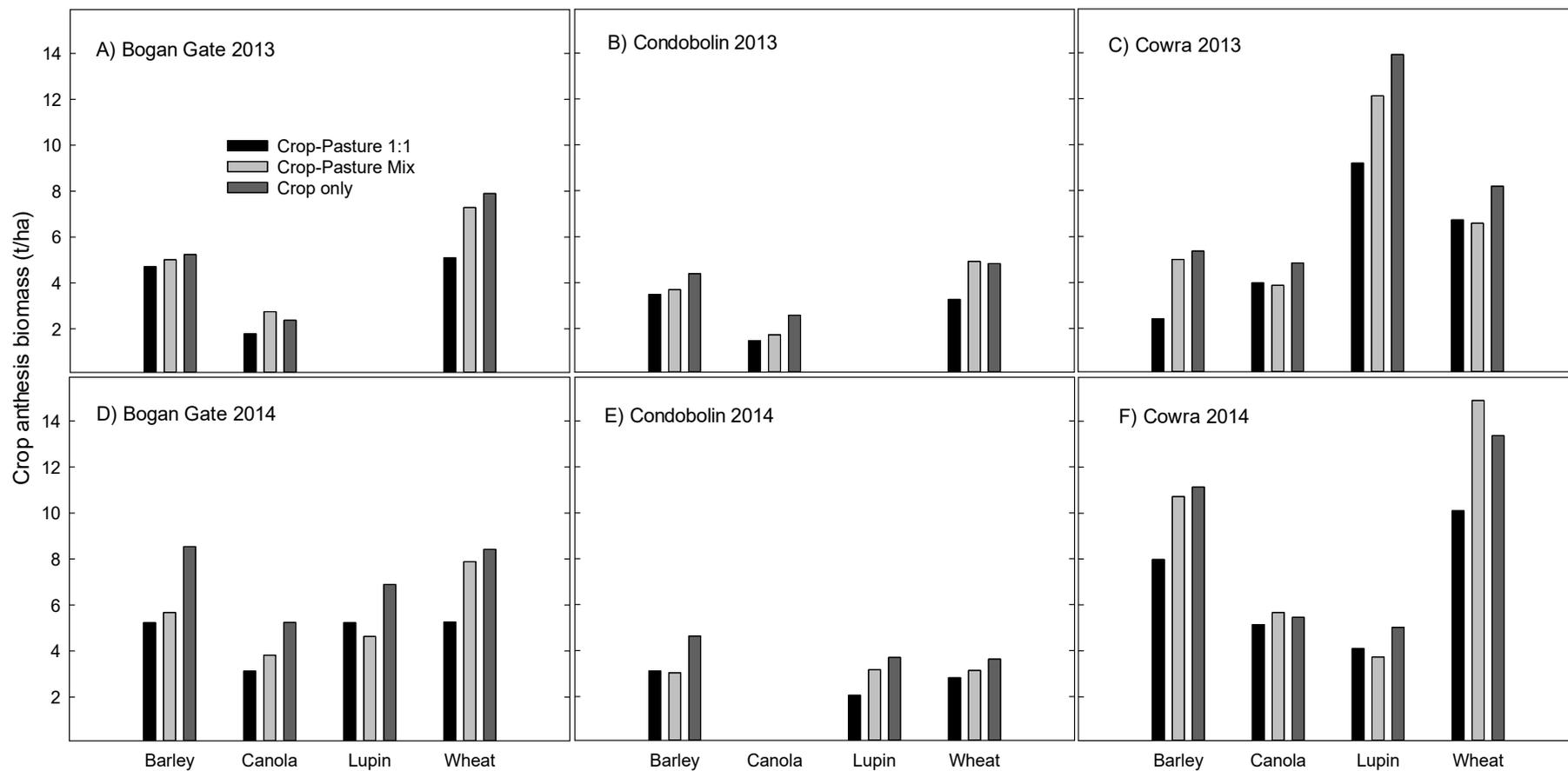
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Supplementary Fig 1. Establishment density (plants/m²) of barley, canola, lupin and wheat sown in different spatial configurations at Bogan Gate (A & D), Condobolin (B & E) and Cowra (C & F) in 2013 (A, B & C) and 2014 (D, E & F).



Supplementary Fig 2. Above-ground biomass at anthesis (t/ha) of barley, canola, lupin and wheat sown in different spatial configurations at Bogan Gate (A & D), Condobolin (B & E) and Cowra (C & F) in 2013 (A, B & C) and 2014 (D, E & F).

Chapter 8

General Discussion

Introduction

Achieving stable and productive mixtures of plant species is a topic of interest to researchers and farmers around the world, applicable to many different production systems in a range of environments. In the rain-fed semi-arid environments of south eastern Australia, there is large application for achieving stable mixtures of pasture species grown in phased rotations with winter crops. There is also a large interest in managing the competition among establishing pastures sown with a cover crop, a common strategy for establishing short-term pastures in this region where the sale of grain from the cover crop in year 1 helps defray the cost of pasture establishment (McCormick *et al.* 2014). Despite decades of research, success in achieving stable mixtures of species is variable (Bowcher 2002, Virgona and Hildebrand 2007) with results often driven by chance of experiencing favourable conditions (Nordblom *et al.* 2017). Agriculture lacks a mechanistic understanding of key processes driving competition in mixed swards and is therefore ill-equipped to develop management strategies that land managers can use to more reliably establish robust and resilient mixtures under a range of seasonal conditions.

Changing row configuration at sowing has long been suggested as a strategy that might be used to achieve more stable and resilient mixtures. Although some studies have used different sowing configurations from time to time, there has been very little evaluation of the effectiveness of the approach. Isolating species in separate drill rows was seen as a practical management strategy due to the low cost and relative convenience to the farmer which, if proven effective, would likely be broadly adopted in a relatively short time-frame (Hayes *et al.* 2017a; Appendix 1). This thesis has reported findings from a large network of field experiments in south-western New South Wales, Australia, to develop a mechanistic understanding of the impacts of changing the spatial arrangement of species in a mixture with a view to inform management decisions.

The thesis examined the effects of different row configuration on biomass accumulation, composition and persistence of pasture mixtures sown to lucerne (alfalfa; *Medicago sativa* L.), phalaris (*Phalaris aquatica* L.) and subterranean clover (*Trifolium subterraneum* L.) grown at five sites over three years, and on lucerne/subterranean clover pastures established under cover crops of wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), canola (*Brassica napus* L.) and lupin (*Lupinus angustifolius* L.) in six experiments at three different sites. Critical to the understanding of

the effectiveness of a new approach to sowing is the legacy of that management, not only for the life of the pasture but on the productivity of subsequent crops in the rotation and on residual soil fertility and function. A detailed sampling of the Wagga Wagga site, supported by crop yield data from two other sites, establishes for the first time the legacy of the pasture drill row and opens some exciting opportunities for further research.

Ecological theory

The final experimental chapter (Hayes *et al.* 2021c; Chapter 7) revealed that changing row configuration of pasture mixtures at sowing often leads to perverse outcomes on productivity due to the negative effect caused by constraining the dominant species to fewer numbers of drill rows. It is worth considering this concept as it applies to short-term pasture phases.

Literature around dominance hierarchies in plant communities is traditionally concerned with long-lived ecosystems containing a large diversity of species. A species in a given community may be ranked by their relative success in competition which is generally reflected in productivity (Whittaker 1965). Total productivity of the ecosystem is the sum of the dominant, or most productive species, plus a lower level of production from subordinate and transient species (Grime 1998). Subordinate species tend to be smaller in stature compared to dominant species and often occupy microhabitats, whereas transient species comprise a heterogeneous group that often exist primarily as seedlings and that do not fit easily into either of the other groupings (Grime 1998).

Mixed pasture swards that are grown in rotations with crops represent a highly disturbed agroecosystem, which only exist at an early stage along the successional gradient determined by the frequency of disturbance events (Crews *et al.* 2016). Diversity of swards is often low, constrained by the small number of species sown by the farmer; the relatively short life (often 3-5 years) of the pasture before the land is returned to several years of annual crop, reducing the opportunity for indigenous or pioneer species to colonise the landscape; as well as the management of the pasture by a farmer, which often includes ongoing weed control with grazing or herbicide. Nevertheless, the same concepts of ecology can be applied to agroecosystems at an early state of succession.

Grime's mass ratio theory would seem fundamental to understanding competition dynamics in pasture phases. The theory holds that ecosystem functioning, including productivity, is largely controlled in the short-term by dominant species (Grime 1998, Sasaki and Lauenroth 2011). Compared to other terrestrial ecosystems such as permanent grasslands or tropical rainforests, a 3-5 year pasture phase is only ever a short-term eco-system highlighting the importance of dominant species in driving productivity of the total sward.

Species dominance

In the experiments in which they were included in this thesis, the winter crops were the dominant species in year 1, representing the largest single component (45-67%) of total biomass (Hayes *et al.* 2021c; Chapter 7). At the end of year 1 all grain from the winter crop was removed, effectively removing the crop component from the sward, leaving the perennial legume, lucerne, to be the dominant species for the remainder of the pasture phase at those sites. In bicultures of perennial and self-regenerating annual species, it is thought that the dominant species would almost certainly be the perennial, assuming all species were adapted to the target environment. Beyond the establishment year, the self-regenerating annual relies on seedling recruitment every autumn to persist, fitting Grime's classification as a transient species (Grime 1998). Reliance on seedling recruitment puts it at a competitive disadvantage when emerging among established perennial species (Dear and Cocks 1997, Dear *et al.* 1998). However, in the establishment year when all species are sown at the same time, it is more difficult to draw this distinction. It would be possible in some environments for the annual species to establish more quickly, due to more vigorous seedling growth, and compete strongly with the perennial species before the perennials got established. Although this was observed with the cover crop species (Hayes *et al.* 2021c; Chapter 7), the annual pasture legume did not out-compete the perennial species in year 1 in any of the field experiments reported in this thesis (Hayes *et al.* 2017b; Chapter 3, Hayes *et al.* 2021c; Chapter 7). Where two perennial species were grown, the question of which species was dominant was determined more by site factors. At sites where soil constraints inhibited lucerne growth, such as Burrumbuttock and Wagga Wagga, phalaris was relatively more productive compared to lucerne, in contrast to other sites where lucerne growth was less constrained (Hayes *et al.* 2017b; Chapter 3). In a separate study in a similar environment, phalaris was the dominant species compared to lucerne but this was largely attributed to low lucerne establishment densities (Hayes *et al.* 2018). At many of the sites described in this thesis, both the lucerne and the phalaris produced similar amounts of biomass implying equi-dominance. Multiple dominant species is a likely outcome in many short-term pastures because there is often not enough time during the pasture phase for one species to dominate the other, assuming both establish successfully and subject to any management regime imposed by the farmer that may bias for or against a particular species.

Managing production and diversity

It was postulated that if sward components were separated initially, interspecific competition would be reduced, improving persistence of all components through enhanced establishment. In the case of the transient species, subterranean clover, the hypothesis was accepted with greater

regeneration where it was spatially separated from other species compared to where it was sown in mixed drill rows, with additional increases in treatments in which it was sown in two out of three drill rows (Hayes *et al.* 2017b; Chapter 3). A similar response was observed in a pilot study where subterranean clover was spatially separated from early-generation perennial wheat lines (Hayes *et al.* 2017c). However, changing row configuration often led to lower total productivity, with effects most obvious in this thesis in the cover-cropping experiments, where there was a large contrast in the early vigour of crop and pasture species (Hayes *et al.* 2021c; Chapter 7). The observed effects were attributed to reductions in the productivity of the dominant species, which by and large, were not associated with reductions in their density (Hayes *et al.* 2021b; Chapter 6, Hayes *et al.* 2021c; Chapter 7). It is likely that containing species to fewer drill rows delayed development of seedlings due to a transient reduction in access to resources, which was rarely severe enough to lead to plant mortality but resulted in reductions in biomass accumulation compared to where the species was sown in a greater number of drill rows.

Taken together, spatial separation of species at sowing was successful, at least to the extent of achieving a better mix of dominant and transient species. However, at the end of this research we might question whether a greater diversity of species is really the true objective, and is diversity warranted if it comes at the cost of lower production? Certainly, there is an advantage in diversity where it means the inclusion of a legume eliminates the need for nitrogenous fertiliser, as is the case in the pasture phase in a rotation (Peoples *et al.* 2012). There is an abundance of literature arguing for and against diverse mixtures, with variable productivity responses reported across a range of field experiments (Jolliffe 1997). Rather than get tied up in what is increasingly an emotive debate, where either outcome is possible and dependent upon the context and environment, a more constructive approach might be to go back to basic principles and try to identify the key factors that will likely achieve both goals of improving sward diversity and productivity, in the context of short-term pasture phases.

If we accept Grime's theory, then total sward production will be driven largely by the dominant species in the mixture. Therefore, to avoid perverse outcomes in production, management strategies need to avoid constraining the dominant species. In the series of experiments described in this thesis, production of the dominant species was constrained where that species was confined to fewer drill rows (Hayes *et al.* 2017b; Chapter 3, Hayes *et al.* 2021c; Chapter 7). Reduced biomass accumulation is a common observation in annual crop monocultures grown at wide row spacings (Scott *et al.* 2013) as wider rows represent a lower total number of drill rows on a given area of land. It may be possible to spatially separate components without reducing the number of drill rows sown to the dominant species, such as by putting the transient regenerating legume in between the drill

rows sown to dominant species. This approach was used by Dick *et al.* (2018) who sowed one of several forage legume species, including lucerne, in between rows of intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkworth & D. R. Dewey). In that instance, drill rows of intermediate wheatgrass were 150 mm apart and there was also a temporal separation of species with the legumes sown eight months after the grass was sown. They reported that legume establishment was successful, achieving 150-200 plants/m², although they did not report the seeding rate used to achieve those densities, other than a target density of 300 plants/m² for each species.

Sowing one species in between drill rows of the other effectively halves the row spacing. This strategy is very likely to maximise productivity as the number of drill rows sown to the dominant species is not diminished and the community of seedlings can make full use of resources above and below ground earlier in their development due to greater coverage. However, it is not clear whether the narrower row spacing (which was 75 mm in the Dick *et al.* 2018 study) is sufficient to reduce competition between the dominant and non-dominant species. This needs to be tested without the confounding effect of temporal separation.

Practical application and future research

Row spacing

In their assessment of row spacing of winter crops sown as pure swards, Scott *et al.* (2013) determined that wider row spacings were generally for the purpose of convenience and practicality rather than to improve yields, *per se*. Improved stubble clearance was the primary advantage in minimum tillage operations, but other factors such as reduced fuel consumption associated with fewer ground-engaging implements and greater speed of sowing operations were also considerations valued by farmers. However, yields generally reduced as row spacing increased, an effect partly attributed to reduced plant density in winter crops. It was only in low rainfall environments where crop yield potential was substantially lower (<1.1 t/ha for cereals) that increasing row spacing beyond 180 mm led to yield increases. In higher rainfall environments, Scott *et al.* (2013) recommended that row spacing should be as narrow as possible to maximise crop yields. The same principle of narrowing row spacing to increase yields, especially under more favourable seasonal conditions, would apply to pastures, notwithstanding the inherent differences in growth habit of pastures compared to winter crops and acknowledging that yield assessment of forages is ongoing throughout the year rather than one assessment of grain yield in spring/summer.

A row spacing as narrow as 75 mm would seem impractical to achieve by doubling the number of tynes on the seed drill. With tynes that close, one would expect the disturbance of soil created by one tyne to interfere with the furrow left by the neighbouring tyne leaving an uneven seed bed and burying seed in some furrows too deep. Clearance of stubble or trash would almost certainly limit the adoption of such a practice on commercial farms (Scott *et al.* 2013), even if the integrity of each furrow could be assured. Temporal separation overcomes this as the initial drill rows have had time to settle and seedlings develop adequately before the second pass. However, the requirement for a second pass is potentially a large disincentive to farmers, adding to the time and cost associated with sowing a mixed sward. An alternative solution might be to broadcast seed in the inter-row at the time of sowing, using scatter plates, or even extra down-tubes that are not attached to a tyne but drop the seed on the surface roughly in a line between drill rows (the so-called inter-seeding approach). On the one hand, broadcasting seed on the soil surface may present a greater risk of establishment failure compared to sowing in drill rows due to poorer seed:soil contact and predation of seed prior to germination (Campbell 1966; 1967). On the other hand, seed of most pasture legume species is small and well suited to shallow sowing, particularly where the act of running the seeder across the field to sow the intervening drill rows likely adds to the texture of the soil in the inter-row, providing crevices in which small seed can lodge to aid germination.

Matrix sowing

Another option is to consider a matrix sowing strategy where drill rows of one species are sown perpendicular to drill rows sown to another species. Preliminary evaluation suggests that this may achieve similar success in the establishment of binary mixtures as separating species in alternate drill rows (Butler *et al.* 2011). As with temporal separation of species, one practical disadvantage of perpendicular rows is that it requires two passes of the seeder, adding to the cost and labour requirements of sowing a new pasture. This approach may also be less suited on undulating country where fields are frequently an irregular shape. Nevertheless, from a competition perspective the method has some merit, in as much as it is another way to spatially separate species, although the degree of separation is not constant with seed of both species inevitably falling in the same spot where the drill rows cross.

One way to evaluate the different sowing approaches from a theoretical perspective is to calculate the area of land that is covered by drill rows. If we assume tyne spacings of 250 mm and an effective row width of 50 mm, conventional sowing practice will result in coverage of just 20% of the land area. A perpendicular arrangement will result in 36% coverage of the land area compared to 40% coverage where seed is inter-seeded between the drill rows, the difference explained by the overlap

where the drill rows cross in the perpendicular arrangement (Figure 1). Alternatively, we can assess the maximum distance from the drill row that would need to be filled in order to achieve full canopy cover, or that roots would need to travel to fully explore the inter-row area. In either case, the maximum distance in the conventional and matrix sowing arrangements is 125 mm, compared to just 75 mm in the inter-seeded configuration (Figure 1). Assuming establishment of legumes is reliable where seed is broadcast between the drill rows, the inter-seeding approach has a number of advantages including a greater coverage of the land area, uniform distance from the dominant species reducing interspecific competition for all broadcast seedlings, and it is achieved with just one pass of the seeder.

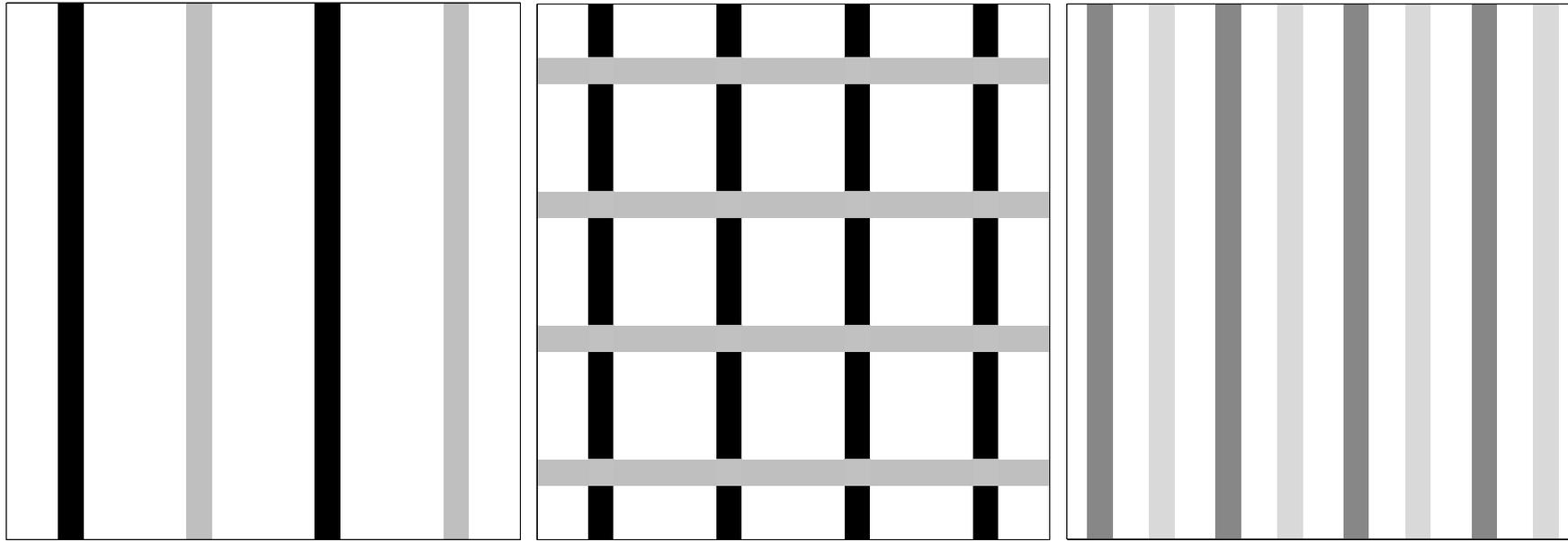


Fig. 1. Conceptual diagram of three different sowing configurations of a binary mixture including (left) alternating drill rows with species 'A' in the black drill rows and species 'B' in the grey drill rows; (centre) matrix sowing configuration with species 'A' sown in the black drill rows in the vertical orientation and species 'B' sown in the grey, horizontal drill rows, and; (right) interseeded sowing configuration with species 'A' sown in the hashed grey drill row only and species 'B' sown also in the hashed grey drill row as well as broadcast onto the soil surface at sowing in between the tynes of the seeder, in the light grey rows. Plots are drawn to scale each representing 1.0 m x 1.0 m in total.

Inter-seeding sowing configuration

Subterranean clover was observed in these experiments to have little competitive effect on dominant species when both were sown in the same drill row (Hayes *et al.* 2017b; Chapter 3, Hayes *et al.* 2021c; Chapter 7). As a general rule, the productivity of a species increased with the number of drill rows in which it was sown. In light of this, it would seem preferable to include some seed of subterranean clover down the drill row mixed with the dominant species, while the remainder of the subterranean clover seed is kept separate from other species and broadcast between the drill rows. Field experiments in this thesis demonstrated that the movement of subterranean clover beyond the initial drill row was slow (Hayes *et al.* 2021b; Chapter 6), attributable to adverse soil conditions for seed burial in a rapidly drying soil surface in spring (Yates 1957). In a phased pasture system where time is clearly limited, it makes sense to include a self-regenerating legume in as many drill rows as possible from the outset to assist it to more effectively colonise all the gaps between the perennial species during periods of the year where soil water and temperature are conducive to growth and can support greater plant density (Hayes *et al.* 2021b; Chapter 6).

Greater plant coverage at sowing is assumed to confer greater benefits to soil health beyond the pasture phase, although this requires validation. In the intensive examination of soil chemical attributes and microbial populations at the Wagga Wagga site, many of the enduring benefits of the pasture drill row were quantified and, in some instances, persisted beyond the first year of crop (Hayes *et al.* 2020; Chapter 4, 2021a; Chapter 5). These benefits were ascribed to plant-driven responses, given the absence of fertiliser in the drill row and the low incidence of movement of plants away from the drill row throughout the pasture phase. Further testing of these effects is warranted using different machinery and on different soil types. For example, it is not clear whether differences in soil organic carbon that were observed at the end of the first wheat crop would exist in a commercial field. The disturbance of the surface soil around the pasture drill row caused by the tynes when the wheat crop is sown may increase mineralisation rates and redistribute nutrients at the soil surface. A priority for future research is to examine whether doubling the plant coverage from 20% to 40% by doubling the number of drill rows confers the same magnitude of benefit to soil microbial populations and fertility as was reported in this thesis (Hayes *et al.* 2020; Chapter 4, Hayes *et al.* 2021a; Chapter 5), or whether those benefits are diluted by the increased number of drill rows and the reduced density of plants within each row. It would also be important to validate that the enduring legacy on soil properties is similar in a furrow left by the tyne assembly of a seed drill as it is between furrows where seed was broadcast on the surface at the time of sowing in an inter-seeding sowing configuration.

If those benefits are validated, there seems reasonable prospect that yield increases in crops following pasture in the rotation will be more obvious than was shown in this thesis. At the Wagga Wagga site it was demonstrated that grain yield of wheat plants growing on the original pasture row was 11% greater than plants growing between rows (Hayes *et al.* 2020; Chapter 4), but when data was aggregated to the plot level and analysed across three sites, there was no significant difference due to pasture treatment (Hayes *et al.* 2021c; Chapter 7). This may have been because there was no difference in the number of drill rows in each treatment, and it made little difference what species was growing in the pasture drill row for those differences on soil fertility to be observed. Nevertheless, a difference in grain size and protein concentration is anticipated in wheat following a grass like phalaris compared to a legume (Dear *et al.* 2004, Hayes *et al.* 2020; Chapter 7). If the same wheat yield advantage existed on 40% of the land area following a pasture sown using an inter-seeded configuration, it seems more probable that aggregated crop yields across the whole area will be greater compared to where those benefits exist only on 20% of the area. Nevertheless, it seems possible to realise crop yield increases by orienting every crop drill row to be sown over every pasture drill row, so long as the action of the tyne when sowing the crop does not undermine the residual benefits of the pasture drill row.

Grazing preference

The question of how row configuration may change the preference of livestock grazing those pastures will be an important consideration in the adoption of novel sowing configurations. Butler *et al.* (2011) postulate that a combination of alternating and perpendicular drill rows provides the greatest prospects for minimising preferential grazing, presumably due to the more even coverage of both species tested when that sowing configuration was used in their study. However, the extent to which this claim can be validated remains uncertain. In a study of grazing dairy cattle, Pembleton *et al.* (2016) showed that intake was generally less on spatially adjacent monocultures compared to where species were sown together in mixed drill rows, suggesting that the increased distance cattle had to walk for the alternative forage may have reduced total intake. The experiments conducted in this thesis did not test grazing behaviour as sites were grazed at very high stocking densities over a short period, unlike most commercial grazing practices. Nonetheless, we might expect that the grazing behaviour of sheep may be different to cattle. At the drier experimental sites, such as Mirrool, where ground cover was low over summer (Hayes *et al.* 2021b; Chapter 6) there was some concern that the absence of the annual legume at that time of year may lead to increased tracking by sheep when grown in alternate rows with lucerne. Further research is warranted to examine the effects of row configuration on grazing behaviour of different livestock.

Conclusion

This thesis establishes a principle, supported by ecological theory, that assists land managers to establish more diverse plantings without sacrificing productivity. The ecological theory is that production in the short term is driven largely by the dominant species in the sward. Spatially separating species at sowing can be an effective strategy at increasing species diversity and need not lead to lower overall biomass accumulation so long as the number of drill rows to which the dominant species is sown is not reduced. Changing row configuration is especially useful in increasing the abundance of subordinate or transient species, which are more vulnerable to competition than dominant species. The self-regenerating annual legume, subterranean clover, benefited from spatial separation from dominant species but despite being a transient species, can be a very important component of the sward and a large contributor to total nitrogen inputs (Peoples *et al.* 2012, Hayes *et al.* 2017b; Chapter 3). There are many potential applications for the principle established here beyond improving composition in phased pastures grown in rain-fed semi-arid environments. Notably, the emerging field of perennial crops research, which aims to grow perennial polycultures (Ryan *et al.* 2018) will inevitably risk yield reductions in the grain crop component of the sward attributable to competition and will require practical approaches such as changing row configuration to maximise grain yields.

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Appendix 1

Prospects for improving the performance of mixed pasture swards by spatially separating components in drill rows*

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Abstract

Maintaining desirable species in a mixed pasture sward is an ongoing challenge for growers in south-west NSW, particularly in lucerne-based pastures. This study examined the prospect of addressing this concern through separating species in drill rows at sowing, a practice change which represents a minimal impost to the farmer. A sensitivity analysis using the ADOPT decision support tool showed that the relatively low level of investment required to try the practice meant that there was potential for relatively rapid adoption provided environmental and/or profitability benefits could be demonstrated. A financial risk analysis determined that, for the scenarios tested on a 1000 ha farm, whole-farm profitability could be increased by between \$523 and \$3162 for every 1% increase in pasture production. Results from field experiments at 5 locations showed that spatially separating species enhanced the persistence of subterranean clover in perennial-based swards but effects on biomass were mixed. Annual pasture production was never significantly greater where subterranean clover was planted in separate drill rows compared to where it was grown in mixed rows with lucerne or phalaris, but in year 1 at three sites biomass decreased by as much as 130% due to the separation of subterranean clover in drill rows. The study also unexpectedly demonstrated an enduring legacy of the original sowing row, with soil microbial populations shown to be twice as abundant under the sowing row compared to between rows in the third year following sowing.

* Hayes RC, Li GD, Gupta VVSR, Hutchings TR, Sandral GA, Swan AD, Peoples MB, Nordblom TL and Llewellyn RS (2017).

Prospects for improving the performance of mixed pasture swards by spatially separating components in drill rows. In: Doing More with Less. Proceedings of the 18th Australian Society of Agronomy Conference, 24-28 September. G. J. O'Leary, R. D. Armstrong and L. Hafner. Ballarat, VIC, Australia, <http://www.agronomyaustraliaproceedings.org/>.

Key Words

Lucerne, phalaris, Subterranean clover, mixture, monoculture, pure sward

Introduction

Lucerne (*Medicago sativa*) has traditionally been sown in mixtures with annual legume species such as subterranean clover (*Trifolium subterraneum*) which increases total nitrogen fixation and winter biomass. Lucerne-based pastures have long been a cornerstone of mixed farming production systems in NSW. Stands are typically maintained for 3-5 years for grazing livestock before they are replaced by annual crops commonly grown for 4-7 subsequent years. The lucerne provides valuable feed for livestock, particularly following episodic summer rainfall events, and fixes significant amounts of biological nitrogen (N) to enhance both livestock and crop production. However, maintaining multiple species in a pasture sward can be challenging, particularly under drier seasonal conditions. The study tested the hypothesis that spatially separating sward components in different drill rows at sowing would enhance the persistence of weaker species, leading to more balanced pasture composition over the life of the pasture.

Adoptability analysis

Altering the spatial arrangement of pasture components at sowing represented a low cost practice change that presented minimal disruption to existing sowing operations. No change to cropping rotation or livestock operations was proposed, but rather the farmer was buying the same seed to put in the same paddock with existing machinery. The only additional impost on the farmer was a modest level of time prior to sowing to reconfigure the seeder such that individual pasture species were delivered down pre-determined sowing tubes. The level of time required by the farmer depended on the particular sowing machinery being used, and the ease with which sowing configurations could be altered. A small number of growers in the Wagga Wagga region and elsewhere had adopted this approach to establishing pastures at a similar time that the EverCrop project began examining the issue. The owner of the equipment in Figure 1 reported that reconfiguring this seeder represented 2-3 hours of his time. The ADOPT model (Kuehne *et al.* 2017) supported the assertion that this practice change would be readily implementable by growers with mixed crop/livestock enterprises, suggesting that near-peak levels of adoption after only 7 years were achievable if relative advantage could be demonstrated. However, a sensitivity analysis revealed that adoption by at least 50% of the target mixed farmer population required at least a

moderate advantage in profit (or equivalent balance of environmental and profit outcomes) compared to existing seeding methods.



Fig. 1. An example of a commercial seeder ordinarily used by a local Wagga Wagga grower to sow pasture components in alternate drill rows. Right: Flows to specific tynes can be blocked allowing different species to flow to separate tynes. In this example, lucerne was delivered from the fertilizer box while phalaris was delivered from the seed box.

Impact of pasture production on whole-farm income

A Sequential Multi-variate Analysis (SMA) was undertaken to assess the likely change in whole-farm profit of increasing pasture yields. Two model farms (1000 ha each) in the Temora (NSW; annual rainfall 520mm) and Rutherglen (Victoria; 590 mm) regions were analysed using assumptions of farm costs established by Hutchings *et al.* (2014). The farms were assumed to grow annual crops in rotation with pastures, and also run a self-replacing merino flock where the older ewes were joined to a terminal sire. The treatments in the analysis were selected to cover the range of practices considered typical in the mixed farming region, and included two sites, four crop rotations, four stocking rates and three pasture yield variations; 96 scenarios in total. Crop yields were estimated using historic rainfall at the sites (Oliver *et al.* 2009) and pasture yields were based on GrassGro outputs for the range of years for which rainfall data was available. All modelled scenarios assumed 80% equity. Financial risk was analysed using Tableau® software (www.tableau.com) which incorporated historical rainfall, commodity prices and yields over 2000 random decadal sequences (Hutchings and Nordblom 2011).

Table 1. Calculation of the incremental change in decadal cash margin (defined as the closing bank balance minus the opening balance) due to changes in pasture metabolisable energy yield, averaged over the 10 and 15 dse/ha stocking rate scenarios

Rotation		Decadal cash margin (\$) with pasture yield			Change in margin/% yield
Crop years	Pasture years	90%	100%	110%	

<i>Rutherglen</i>					
1	4	-\$1 727 127	-\$1 385 510	-\$1 121 808	\$3 027
1	8	-\$1 896 239	-\$1 590 169	-\$1 263 840	\$3 162
5	4	-\$175 276	-\$149 064	-\$70 673	\$523
5	8	\$236 696	\$287 030	\$355 176	\$592
<i>Temora</i>					
1	4	-\$1 122 061	-\$1 570 744	-\$754 683	\$1 837
1	8	-\$218 470	-\$726 442	\$102 138	\$1 603
5	4	-\$383 176	-\$313 881	-\$224 887	\$792
5	8	\$357 611	\$405 343	\$520 030	\$812

Crop and pasture yields and the subsequent energy that could be harvested by livestock varied substantially with time, driven largely by the variation in rainfall at the sites. For example, pasture metabolisable energy yield was calculated to be > 160 000 MJ/ha in 1973, and as low as 21 000 MJ/ha in 2006. Results showed that there was little difference in the decadal cash margin between the two sites. The optimum stocking rate for all rotations was shown to be 10-15 dse/ha. The scenario with the best financial outcome was the 5 crop- and 8 pasture-years rotation (~60% pasture to 40% crop). A series of simulations was run at both sites, varying the pasture grazing energy yield by 10% either side of the mean. Results showed that increasing pasture yield had more impact on the minimum decadal cash margin, than on the average or the maximum values. This indicates that the main benefit of increasing pasture yield may be due to a reduction of the size of cash losses at the whole-farm level; a valuable trend which may not be reflected in a change in the mean values. Over the range of scenarios tested, a 1% increase in pasture yield led to an annual increase of \$523-\$3162 in whole farm profit (Table 1).

Pasture production and persistence

Five field experiments were established in 2012 in south-west NSW to test the response of lucerne-based pastures sown in different spatial configurations with phalaris (*Phalaris aquatica*) and subterranean clover. Full details of experimental methods and results are described by Hayes *et al.* (2017). There were few consistent effects on total pasture biomass attributable to spatial configuration providing no clear signal of a universal benefit of changed spatial configuration, reflecting site-specific or season-dependant nature of results. However, averaged across sites, subterranean clover regeneration at the end of the experimental period was 29% higher and total cumulative biomass was 13% higher where subterranean clover was sown in alternate drill rows with lucerne compared to where both species were mixed together in every drill row. Increases in

cumulative annual dry matter production were up to 16% in phalaris/subterranean clover swards, and up to 10% in lucerne/subterranean clover swards where the two species were planted in alternate rows compared to where they were planted in mixed row configurations. However, reductions in cumulative annual dry matter of the same treatments at different times and different sites were as high as 68%. This field study demonstrated that whilst subterranean clover persistence was consistently enhanced in lucerne swards where the two species were spatially separated, large increases in total biomass could not be anticipated, and decreased biomass was a possibility.

Soil microbiology

Changes in soil health as indicated by increased microbial populations and function (Doran and Zeiss 2000) would be of key interest to farmers as this directly impacts upon long term productivity and resilience. At the Wagga Wagga site, three contrasting treatments were sampled 27 months after pastures were sown, to quantify differences in the abundance of soil bacteria and fungi in the surface 0.1 m, both under and between particular sowing rows (Hayes *et al.* 2017). The treatments included pure subterranean clover (Subclover_Mono) and clover grown in alternate rows with either lucerne (Lucerne_Subclover_1:1) or phalaris (Phalaris_Subclover_1:1). Samples of moist soil were taken in September 2014 and microbial abundances were determined using group-specific primers (bacteria 16S rRNA; fungi – ITS region), following established methods (Gupta *et al.* 2014).

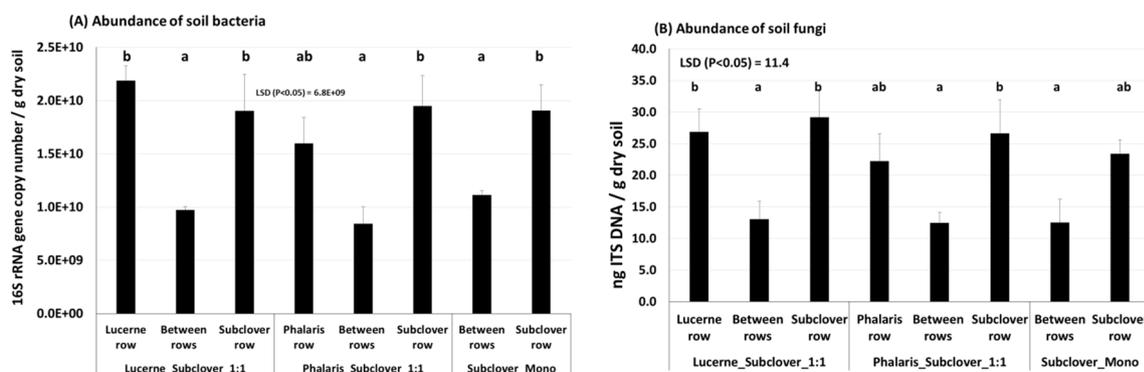


Fig. 2. Localized spatial variation in the abundance of soil bacteria (A) and fungi (B) in pure and mixed pasture systems.

The abundance of bacteria and fungi were significantly higher in soil directly beneath the original sowing row for all species compared to between rows (Figure 2). This result was not expected in mature pasture stands, suggesting that even in the third year of pasture, plants may not be utilizing the full soil resource in terms of biologically mediated functions and the rhizodeposition effects are mostly confined to the original sowing row only. Total abundance of bacteria and fungi was not significantly different according pasture species that were sown on the particular row, although the

variation in community composition of bacteria and fungi between pasture species is still under investigation. Early findings suggested that sowing configuration has an enduring legacy on soil microbiology. Further research is required to understand i) the likely impact of this legacy on the long-term production capacity of the soil, and ii) if this legacy can be utilised more effectively to increase profitability of mixed farming/grazing systems.

Discussion and conclusion

An examination of the practice of spatially separating components of mixed pasture swards in drill rows was undertaken as a means of improving species co-existence as previously proposed by (Boschma *et al.* 2010). Implementing the practice represents little impost on the farmer. The ADOPT decision support tool demonstrated that it would only take a relatively short time to achieve peak adoption providing the practice could be shown to have consistent net relative advantage. A sensitivity analysis was undertaken within a broader Sequential Multi-variate Analysis of farm financial risk and showed that for every 1% increase in pasture biomass, whole farm profit would increase by between \$523 and \$3162 based on two 1000 ha model farms. Initial investigations in the field showed that spatially separating subterranean clover from either lucerne or phalaris improved annual legume persistence and led to biomass increases of up to 16%. However, results were site- and season-dependant and there were other instances where spatially separating components actually led to decreases in annual biomass production of up to 68%. The profit-driver of this practice change is therefore perhaps not strong enough to anticipate widespread farmer adoption without significant environmental benefit. Nevertheless, the fact that some farmers have already adopted the practice suggests that they recognise some benefit of the practice, which could be associated with better annual legume persistence leading to higher inputs fixed biological nitrogen. An unexpected finding from the field experiment was the legacy of the original sowing row on soil bacteria and fungi 27 months after the pasture was sown, where the abundances of these soil microbes in the inter-row area were shown to be only half the levels observed beneath the original sowing row. This finding warrants further investigation to determine its full significance on the production capacity of the soil, such as to determine the functional composition of these microbial populations, and to determine what, if any, associated differences can be observed in the chemistry and nutrition of the soil. This component of research remains ongoing.

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