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Livestock in diverse cropping systems improve weed management and sustain yields whilst reducing inputs

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Keywords: weed management, crop rotation, crop diversity, integrated crop-livestock systems, selection pressure, grazing, forage legumes

Abstract

1. Intensive cropping systems select for a low diversity of weeds tolerant of chemical control, leading to persistent weed-crop competition and declining biodiversity. Crop rotation can mitigate this by introducing variable filters on the weed community through increasing management diversity. In this study we investigate the effect of integrating livestock into no-till crop rotations to complement chemical weed control.

2. We analysed twelve years of weed seedbank data from a trial of eight rotation systems with different crop sequence diversities, of which four included grazed forage phases. Linear mixed models and ordination were used to assess how weed abundance, diversity and community composition responded to management filters, defined in terms of levels of disturbance strength and diversity (grazing and herbicides), and resource availability and diversity (inorganic fertilisers, legumes and manure).

3. Grazed rotation systems had less herbicide applied than ungrazed rotation systems, and had the lowest weed abundance and highest weed diversity. Herbicides and grazing apply contrasting selection pressures on weeds, and this combination was more effective in reducing weed pressure than increasing herbicide quantity or mode-of-action diversity. Lower resource availability and higher nitrogen source diversity in grazed systems may have further reduced weed abundance and promoted diversity.

4. Crop sequence diversity also reduced weed abundance and promoted weed diversity, indicating that variable crop-weed interactions can enhance weed management. In addition, yields in the main cash crop (wheat) were highest where crop diversity was highest, regardless of whether the system contained grazed phases.

5. *Synthesis and applications.* Diverse rotation systems produced high yields, and the inclusion of grazed forage phases maintained these yields at lower applications of herbicides and fertilisers: integrated livestock can therefore improve the sustainability of no-till systems. The role of grazing as a filter imposing a contrasting selection pressure to other weed control options could be further explored to improve weed management in different farming systems.

1. Introduction

In recent decades, farming systems have become increasingly specialised to produce a small number of crops on large scales in short rotations, and to separate crop production from livestock production. This has been facilitated by the introduction of high yielding cultivars in a few major crops, inorganic fertilisers, pesticides and specialised equipment. However, the long-term prospects of this 'Green Revolution' are in doubt: the environmental impacts and the tendency of such systems to select for a small number of highly injurious pests, weeds, and diseases, have led to recent calls for the re-diversification of cropping systems as part of the drive for 'sustainable intensification' (Pretty and Barucha 2014). Increasing cropping system diversity can increase both agricultural productivity and sustainability (Isbell et al 2017), and diverse crop rotations in particular have been shown to improve soil fertility, suppress pests and diseases, support beneficial biodiversity, and stabilise incomes (Davis et al 2012, Wezel et al 2014). These benefits may be further enhanced by re-integrating cropping and livestock systems (Sanderson et al 2013, St-Martin et al 2017).

Long-term experiments that investigate the functions of diversity across whole farming systems make an important contribution to re-diversification, by enabling the study of processes that manifest over decadal time scales, such as weed community dynamics (Paul et al 1998, Storkey et al 2016). Previous findings indicate that the multiple benefits of crop rotations can result from the different ecological and economic properties of different crops, but are often also driven by variation in management associated with different crops (Davis et al 2012, Gaba et al 2013, Wezel et al 2014). Intensive cropping systems lacking in management variation tend to have weed communities dominated by only a few species with strongly ruderal traits that confer advantage in resource-rich, frequently disturbed environments (Storkey et al 2010, Storkey et al 2012, Reich 2014), and herbicide resistant species are also common (Neve et al 2009, Mortensen et al 2012). This indicates that

consistent management actions reduce weed diversity, but fail to suppress species tolerant to those actions. Weed-crop competition therefore persists, despite substantial investment in weed control, whilst the ecosystem services offered by a diverse weed community are lost (Petit et al 2015, Gaba et al 2016).

Crop management actions can be interpreted as filters on the weed community, allowing species that possess traits conferring tolerance to the disturbances and conditions imposed by management to thrive, and limiting the survival of those that do not. Varying management between years alters the pattern of this selection pressure each year, reducing the chance that any single weed species is driven to extinction, but increasing the chance that all species would encounter limits to their survival and reproduction at some point (Booth and Swanton 2002, Navas 2012). This also limits the opportunities for weeds to adapt to a consistent set of conditions, as has occurred with the evolution of herbicide resistance in response to the frequent cultivation of a limited number of crops reliant on a small range of herbicide active ingredients (Neve et al 2009, Mortensen et al 2012).

Several studies have shown that crop rotations involving differences in the techniques and timings of sowing, harvest, soil preparation and herbicide use are effective for weed management (Anderson 2015, Blackshaw et al 2015, Petit et al 2015). However, it remains unclear whether crop rotation itself is sufficient, if different crops are not associated with different management (Smith and Gross 2007, Mortensen et al 2012). In this context, a major limitation of the recent spread of no-till cropping practices is the loss of tillage as a weed control option, and the reliance of these systems on herbicides. One option to increase the diversity of weed selection pressure in no-till systems is to integrate livestock, by adding grazed forage crop phases to the rotation. This practice is widespread in some regions of the world and appears profitable for farmers, but remains relatively understudied with regard to weed management (Sanderson et al 2013). Grazing would be expected to directly suppress weeds, and in addition, the combination of a forage legume and livestock manure

may further enhance weed diversity through increasing nitrogen resource diversity (Smith et al 2010).

In this study, we compared long-term weed seedbank trends between rotation systems with different crop sequence diversities, and between crop-only systems and integrated crop-livestock systems. Ungrazed systems with low crop diversity were subject to agrichemical-intensive management, resulting in a strong consistent disturbance induced by herbicides, and high resource availability from fertilisers. In contrast, diverse systems with livestock incorporated grazing, legumes, herbicides and fertilisers, resulting in more diverse disturbances and nutrient sources. By comparing the different rotation systems, we thus explored the following hypotheses:

- (1) The diversity of management filters (disturbance diversity and resource diversity) reduces weed abundance and increases weed diversity.
- (2) The strength of management filters (disturbance intensity and resource availability) increases weed abundance and reduces weed diversity.

2. Methods

2.1 Trial location, layout and timing

This study used weed seedbank data from the Langgewens Long-Term Crop Rotation Trial, which investigates the agronomic performance of eight different crop rotations under conservation agriculture practices. The trial is located in South Africa's Western Cape Province (33°17'0.78"S, 18°42'28.09"E; Fig. 1). The site receives an average annual rainfall of 376mm, with approximately 80% received during the winter months. This constrains regional production to one crop per year, sown in April and harvested in November, with a

fallow period over summer. The trial began in 1996, but weed data was only systematically collected across all systems since 2005, thus the twelve years in this study span 2005 - 2016. The eight systems are each replicated twice in a randomised block design, and within each replication all crop types are planted each year in the order of the specified rotation (Table 1). See the appendix for a full explanation of the trial design. Plot sizes vary between 0.5 and 2 ha, depending on the system diversity and whether the system is grazed, but the data used here is based on weed seeds collected in the same amount of soil from each plot, so plot size would not affect the sample. The use of seedbank data allowed us to quantify trends without the confounding effect of stochastic processes that can influence the emerged weed flora in any given year.

Crop species included in the trial are wheat (*Triticum aestivum*), canola (*Brassica napus*), lupins (*Lupinus angustifolius*), and annual self-regenerating medic species (*Medicago truncatula* and *M. polymorpha*) and white clover (*Trifolium repens*) (Table 1). Wheat and canola function as cash crops, lupins as ungrazed cover crops (with seeds harvested for income), and annual self-regenerating medics and clovers as forage crops grazed by sheep (*Ovis aries*), at a stocking rate of four sheep ha⁻¹ (standard local practice; Basson 2017). Sheep are moved onto the forage crops when the medic and clover pastures begin to establish in April or May (these regenerate each year but are sprayed off in cash crops). In system H, sheep are kept aside in additional pastures to forage on saltbush (*Atriplex nummularia*) for approximately six weeks until the annual medic/clover mix has reached at least 90% groundcover. Sheep also graze winter crop residues over the summers in systems E-H, and are occasionally used for short periods (four to five days) toward the end of the summer fallow period in the ungrazed systems, as their trampling can break up high residue loads to ease planting. This is done before the first rains and prior to planting, and the lack of summer rainfall in the region means that few, if any, weeds are present at this time and thus briefly introducing sheep in this way would have minimal impact on weeds in otherwise ungrazed systems. All rotation systems are managed according to local best

practices and industry recommendations, resulting in variation in agrichemical use between rotation systems and over time (Fig. 2). From 1996 to 2001, the trial was under minimum-tillage (a disc harrow was used to prepare the seedbed), and since 2002 the trial has been under no-till practices with a tine planter.

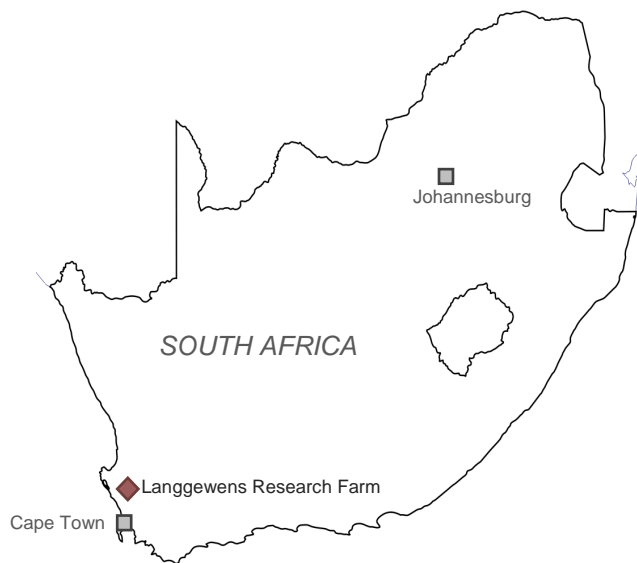


Figure 1: The location of Langgewens Research Farm in the Western Cape, South Africa.

Table 1: The composition of the crop rotations in the eight different rotation systems included in the Langgewens Long-Term Crop Rotation Trial. Crop phases marked with (G) were grazed by sheep.

Code	Rotation system
A	Wheat – Wheat – Wheat – Wheat
B	Wheat – Wheat – Wheat – Canola
C	Wheat – Canola – Wheat – Lupins
D	Wheat – Wheat – Lupins – Canola
E	Wheat – Medic (G) – Wheat – Medic (G)
F	Wheat – Medic/clover mix (G) – Wheat – Medic/clover mix (G)
G	Medic (G) – Wheat – Medic (G) – Canola
H	Wheat – Medic/clover mix* (G) – Wheat – Medic/clover mix* (G) *with saltbush pastures to rest medic/clover pastures

2.2 Data collection

2.2.1 Weed seedbank samples

Seedbank samples were collected in late March or early April prior to planting each year. From each plot, 80 soil cores of 105 mm diameter and 5 cm depth were combined to form a single sample. The experiment is a no-till system so weed seeds were assumed to be concentrated in this surface layer. Directly following sampling, the soil was placed in 400x250mm trays in a layer approximately 20mm thick over sterilised river sand, under shade-nets with regular irrigation to promote germination. Seedlings that emerged were counted with removal between two and four times until September. Occasionally seedlings could not be identified; these constituted 4.3% of the seedlings observed and were not included in the dataset. ‘Volunteer’ seedlings belonging to the crop species used in the trial

were also not included. This direct germination method was used rather than a seed extraction method due to the lower risk of under-representing species with small and light-coloured seeds (Gross 1990). Both methods are suitable for detecting seedbank changes in response to agricultural management (Ball and Miller 1989).

During the twelve-year timeframe each plot completed three full four-year rotations, allowing the seedbank to be assessed at the level of the whole rotation with three time periods. Seedling counts were averaged across each four-year rotation period: 2005 to 2008 = Period 1, 2009 to 2012 = Period 2, and 2013 to 2016 = Period 3. 'Weed abundance' subsequently refers to the average number of seedlings per year within each period. 'Weed diversity' is the average species diversity of seedlings per year, calculated using Fisher's log series alpha. This diversity index is insensitive to differences in abundance (Magurran 2003), and was selected due to large differences in weed abundance between treatments.

2.2.2 Agronomic data

The amount of fertilisers and herbicides applied to each plot were aggregated to a total amount per hectare over each four-year period. Herbicide quantities were standardised within each active ingredient (to the proportion of the maximum dose of that ingredient applied in the trial) to take account of differences in potency among different active ingredients. Wheat was harvested each year with a combine harvester, and the yield (wet grain weight standardised to 14% moisture) for each plot was converted to the proportion of the average yield within the trial for that year. This accounted for inter-annual yield variation in response to climate variables such as rainfall, allowing any consistent effect of rotation system on yield to be identified across different years.

2.3 Data analyses

All analyses were undertaken in *R* Version 3.4.3 (R Core Team 2017), using the packages *lme4*, *afex*, *lsmeans*, *effects* and *vegan*. Prior to analyses, weed abundance was converted to the natural logarithm of the abundance plus one.

2.3.1 Differences in weed abundance, weed diversity, and wheat yield between systems

Differences in weed abundance, diversity and wheat yield between rotation systems were investigated using linear mixed regression models. As fixed effects, the models for weed abundance and diversity included the main effects and interaction terms for rotation system and period. The wheat yield model included only rotation system, as variation between periods had been accounted for by using yields standardised within each year. Plot was included in all models as a random effect to account for repeated measures in the same plot over time. P-values for the significance of fixed effects were calculated using parametric bootstrapping, one of the most reliable methods for mixed models (Halekoh and Højsgaard 2014). This approach involves comparing differences between the full model and sub-models, and thus does not generate P-values for each level of a factor, only whether the effect of the factor is significant overall. Tukey's pairwise comparisons were used to assess differences between the different rotations and periods in each model. Differences could thus be assessed between low and high crop diversity within either the ungrazed or grazed systems, or between grazed and ungrazed systems or either lower or higher crop diversities.

2.3.2 Differences in weed abundance and diversity in response to filter strength and filter diversity

The same modelling approach as above was employed to explore how weed abundance and diversity responded to differences in crop sequence diversity, herbicides, fertilisers, and grazing. These variables were used to explore the two hypotheses of this study regarding filter strength and filter diversity. The presence or absence of grazing and the number of

herbicide mode-of-action groups used indicated the diversity of disturbances, while the amount of herbicide applied (grams of active ingredient per hectare) represented the strength of the herbicide disturbance. Grazing pressure differed slightly in strength only in system H, where sheep grazed the medic/clover pastures for approximately 20% less duration each season, but otherwise all grazed systems had two forage phases with four sheep ha⁻¹. To minimise the complexity of the analyses, grazing was included as either 'present' or 'absent', but the reduced duration in system H was noted when interpreting the results.

The number of nitrogen sources available represented resource diversity: these were synthetic nitrogen fertiliser, nitrogen released from legume crops, and nitrogen circulated to soil through sheep manure and urine. The amounts of synthetic nitrogen, phosphorus and potassium fertiliser applied were considered indicators of maximum resource availability. The trial is managed to provide adequate nutrition to each crop through fertilisers, crop residues and/or livestock manure, and thus the overall quantity of nutrients that become available over the season within each system can be assumed to be similar. However, research suggests that nutrients from organic sources such as crop residues and manure are released gradually over the season, whilst synthetic fertilisers provide a flush of nutrients at the time of application, and thus a high peak of nutrient availability (Poudel et al 2002, Crews and Peoples 2005). Furthermore, this peak would occur early in the season when the majority of fertiliser is applied, when crop seedlings are too small to efficiently capture nutrients and competition imposed on weeds would be weak. A higher maximum nutrient availability resulting from higher fertiliser applications is therefore expected to increase weed abundance and reduce weed diversity, whereas the longer duration but lower maximum resource availability resulting from nitrogen resource diversity is expected to be associated with fewer weeds with a greater diversity.

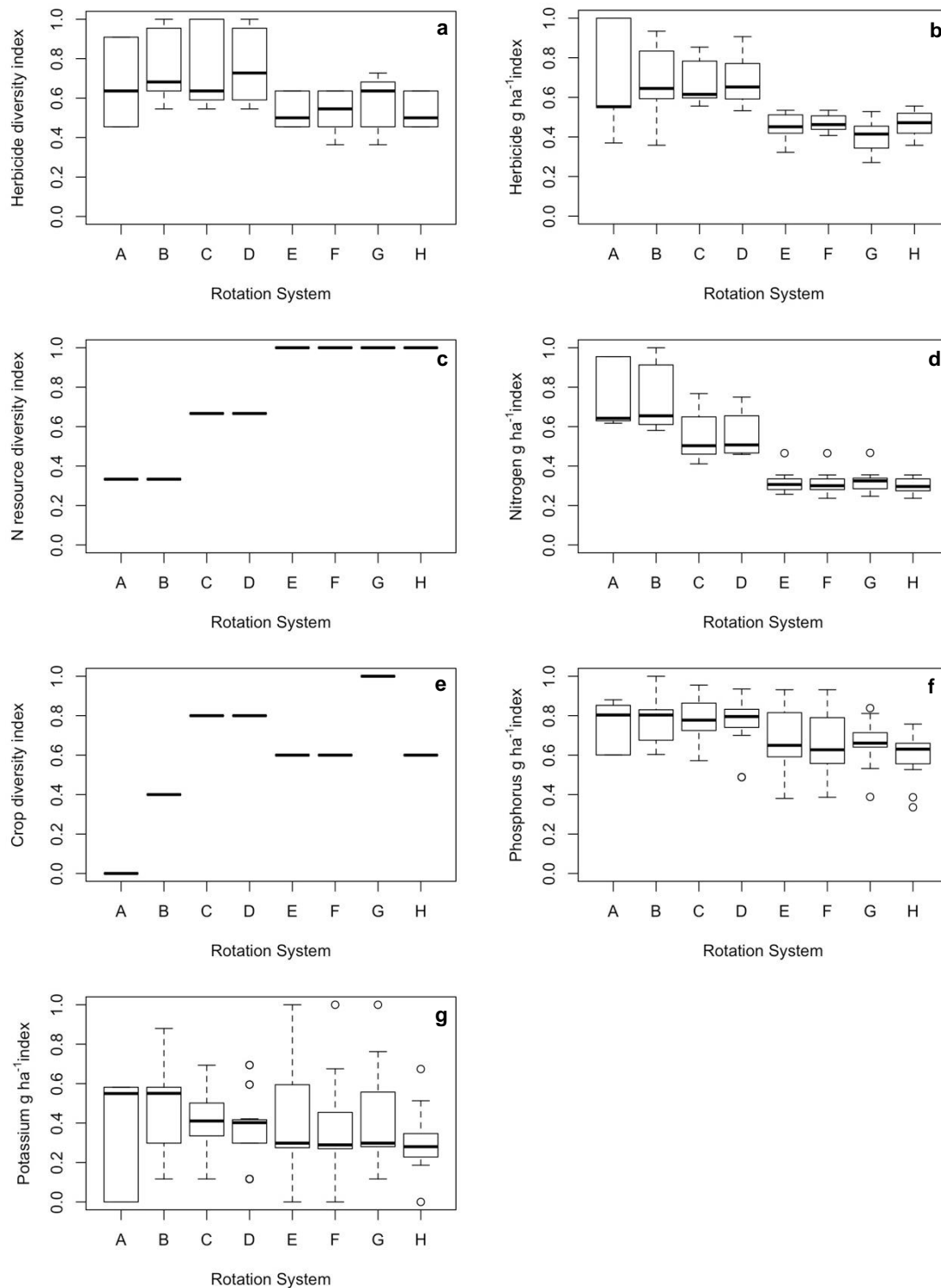
Crop sequence diversity was also included to assess whether it had an independent effect on weeds in addition to the disturbance and resource variables. Within-year diversity (i.e. the medic/clover mix in systems F and H) was not assessed, nor were the saltbush in system H, as these perennial shrubs were located on separate plots outside the rotation.

Indices for each of the above variables were calculated for each plot in each period and scaled to between 0 and 1 (Table 2; Fig. 2) to standardise the different metrics of disturbance. All variables, except for crop sequence diversity, were collinear (detected through high variance inflation factors) and could not be included in the same model. Different models were therefore constructed for each collinear variable, and contained period, crop sequence diversity, the variable of interest, and the interaction between crop diversity and the variable of interest. No interaction with period was included as there was no significant interaction between rotation system and period in the previous set of models, thus it was not logical to expect period to interact with management differences between rotations. Plot was again included as a random effect. The results of these models were interpreted by investigating a) whether each variable was significant using the P-values calculated by parametric bootstrapping, and b) whether any models had a better fit than others, by comparing their Akaike Information Criteria (AIC). All linear mixed models were fitted using maximum likelihood, as opposed to restricted maximum likelihood, to ensure that parametric bootstrapping and AIC comparisons were valid.

Table 2: A description of the indices of management and resource variables investigated in relation to weed abundance and diversity. All indices have been scaled to between 0 and 1 to make the model effect estimates comparable; this was done by expressing each value of each index as a proportion of the maximum value.

Variable	Description
Crop sequence diversity	The number of non-wheat years multiplied by the number of non-wheat crop types in each rotation system.
Herbicide AI (g ha ⁻¹)	The amount of active ingredient (AI) (g ha ⁻¹) applied to each plot within each four-year period (standardised by active ingredient)
Herbicide diversity	The number of different herbicide mode-of-action applied to each plot within each four-year period
N / P / K (kg ha ⁻¹)	The total amount of fertiliser (kg ha ⁻¹) applied to each plot within each four-year period. Separate indices were calculated for nitrogen (N), phosphorus (P) and potassium (K).
Nitrogen source diversity	The number of different types of nitrogen resource (nitrogen fertiliser, legumes, and sheep manure/urine) available within each rotation system
Grazed/ungrazed	Whether the rotation system included sheep forage phases or not (all systems with sheep had two forage phases).

Figure 2: Variation in management and resource indices among rotation systems for all plots in each period: (a) herbicide active ingredient (AI, g ha⁻¹), (b) herbicide diversity, (c) crop diversity index, (d) nitrogen resource diversity, (e) nitrogen fertiliser (kg ha⁻¹), (e) phosphorus fertiliser (kg ha⁻¹), and (g) potassium fertiliser (kg ha⁻¹). Box plots indicate the median, interquartile range, and minimum and maximum (open circles are points more 1.5 times the interquartile range from the median).



2.3.3 Weed community composition

A non-metric multidimensional scaling (NMS) ordination based on the Bray-Curtis distance measure was employed to explore variation in weed community composition between each plot in each period. An NMS is an unconstrained ordination technique, and was chosen over a constrained ordination approach as constrained ordinations are based on linear regression, and would thus have been unreliable due to the collinearity among management variables.

3. Results

3.1 Weed diversity and abundance and wheat yield in different rotations

Rotation system had a significant effect on all three responses investigated: weed abundance, weed diversity and relative wheat yield (Table 3). Pairwise comparisons indicated that all rotations containing grazed forage phases (E-H) had significantly lower weed abundances and higher weed diversity than ungrazed rotations (Fig. 3). The shorter grazing duration in system H did not have an effect, as weed abundance and diversity in system H were not significantly different from that of systems E or F. System G, the grazed rotation with the highest crop diversity, had a significantly lower weed abundance and higher weed diversity than all other rotation systems, excluding E. However, the pairwise comparisons did not otherwise indicate that more diverse rotations had lower weed abundance or higher weed diversity than less diverse rotations, within either the non-grazed (A-D) nor grazed systems (E-F).

Weed abundance was also significantly affected by time period, and increased slightly from Period 1 to Period 3 (Fig. 3b); this may have been in response to rainfall differences

between periods, or may indicate evolution of herbicide resistance amongst weed populations. For relative wheat yield, the monoculture wheat system A had a significantly lower yield than the four most diverse rotations (C, D, G, and H), and there was a general trend that wheat yields increased with crop diversity (Fig. 3). Absolute wheat yields (not standardised within each year) were also explored for any obvious trends over time, but the inter-annual variation in response to rainfall was too great to identify any trends in absolute yields (results not shown).

Table 3: Fixed effect estimates and P-values from the models of weed abundance, weed diversity and relative wheat yield in response to rotation system and time period (see also Fig. 3). Time period was not included in the yield model (variation over time was accounted for by using yield standardised within each year). Estimates for Periods 2 and 3 and relative to Period 1. The model estimates for the interaction between each level of period and rotation are not shown, as the interaction was not significant (NS).

	Abundance		Diversity		Wheat yield	
	Estimate	P-value	Estimate	P-value	Estimate	P-value
System B	1.47		-0.45		-0.17	
System C	1.1		-0.3		-0.07	
System D	0.92		-0.23		0.05	
System E	0.82	<0.001	-0.19	<0.001	0.04	0.027
System F	-1.03		0.42		0.03	
System G	-0.72		-0.06		-0.03	
System H	-2.01		0.77		0.08	
Period 2	-0.22		-0.03		-	-
Period 3	0.04	0.033	-0.02	0.654	-	-
Interaction (rotation x period)	NS	0.155	NS	0.138	-	-

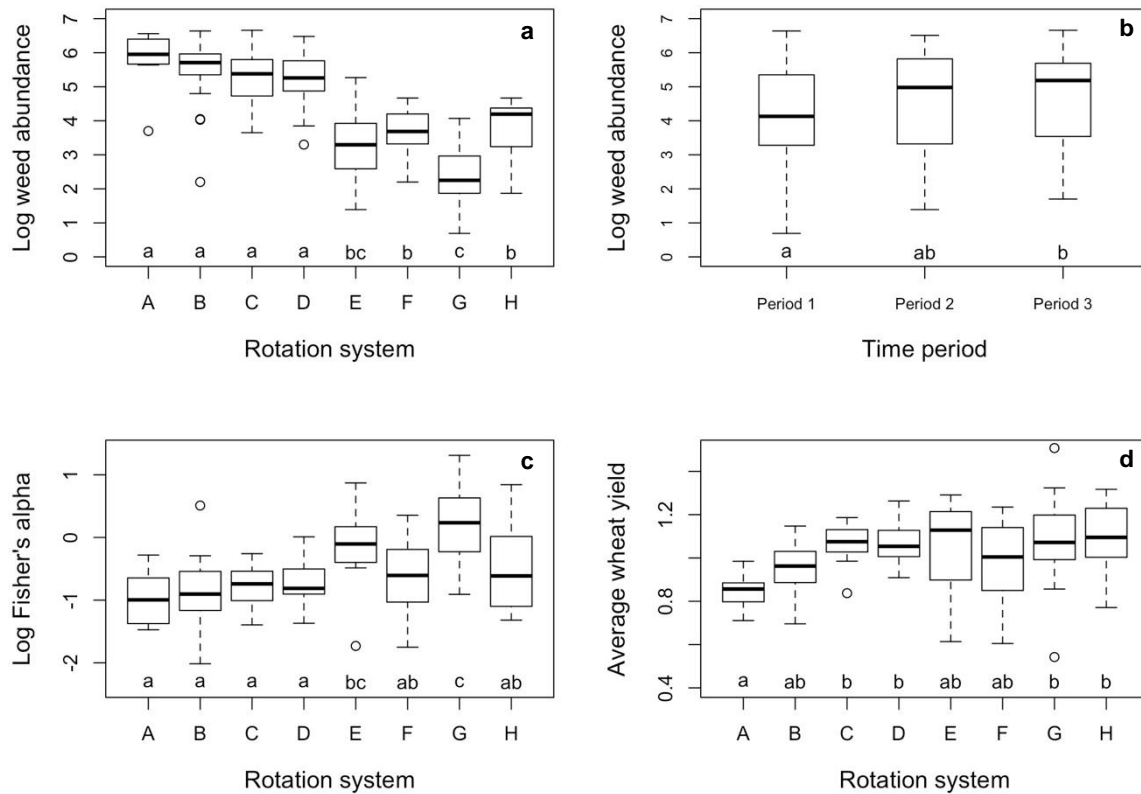


Figure 3: Relationships between weed abundance, weed diversity, wheat yield and rotation system and time period: (a) log weed abundance in the different rotation systems; (b) log weed abundance in the different time periods; (c) Fisher's log series alpha diversity index of weeds in the different rotation systems; (d) relative wheat yield in the different rotations. Categories with significant pairwise differences ($P < 0.05$) do not share letters along the base of the plot. Refer to Table 1 for rotation system crop sequences, and to Table 3 for model statistics.

3.2 Weed diversity and abundance in relation to management and resource diversity and intensity/availability

Of all the management and resource indices, only crop diversity had a significant main effect on weed abundance and weed diversity (Table 4). However, grazing, herbicide amount, nitrogen availability and nitrogen source diversity all had significant interactions with crop diversity (Table 4). The lack of a significant main effect may be due to the experimental design in relation to the variables tested. For example, there were no grazed systems at low crop diversities, and thus the model had no information with which to estimate an effect of grazing in the absence of crop diversity. The significant interaction indicates that grazing affected the relationships between crop diversity and weed abundance and diversity: Figure 4 illustrates that as crop diversity increases, weed abundance decreases; but if the rotation system contains sheep, then weed abundance decreases further for a given increase in crop diversity (Table 4; Fig. 4, Figs 5a and 5b). The same trend exists for herbicide amount, nitrogen source diversity and nitrogen availability. Herbicide group diversity, and phosphorus and potassium fertiliser availability, were not significantly associated with either weed abundance or diversity (results not shown).

Overall, the model results indicate that higher weed abundances and lower weed diversity occurred in ungrazed rotations and were associated with decreased crop diversity, increased quantities of herbicides and higher maximum nitrogen availability, and reduced nitrogen source diversity (Table 4, Fig. 4, Fig. 5). The individual contribution of each variable to the variance in weed abundance and diversity could not be attributed, given the collinearity between them. However, the AIC is lowest for the model containing crop diversity and grazing (Table 4), suggesting that these are the strongest drivers of differences in weed abundance and diversity.

Time period had a significant effect in some models, although the effects were small compared with the effects of the management and resource variables. The models suggest either a small decrease or increase in weed abundance in period 2 from period 1, then a larger increase in period 3 (see also Fig. 3b). Both periods were typically associated with a small decline in diversity, although this was significant only in the model including the total N fertiliser index. Rainfall also increased in Period 3, possibly explaining this trend (results not shown).

3.3 Weed community composition in relation to rotation system, time period, management and resource indices

Ten weed species emerged from the seedbank samples over the twelve years (Table 5), excluding volunteer crop seedlings and the occasional unidentifiable seedling. *Lolium* spp., (a hybrid complex primarily between *L. rigidum* and *L. perenne*; Ferreira et al 2001) was by far the most dominant weed in the system: on average 77% of seedlings in each sample were *Lolium* seedlings (Table 5).

A two-dimensional NMS solution was selected to represent variation in the relative frequency of these species across the trial. Two dimensions reduced stress to an acceptable level (ordination stress = 0.17 and non-linear $R^2 = 0.97$), and whilst the addition of a third dimension reduced stress further (to 0.12), it did not alter any trends shown, and was thus omitted to conserve interpretability. The ordination indicates that ungrazed systems were associated with consistently high abundances of *Polygonum aviculare* and *Lolium* spp., while species composition varied more within grazed systems (Fig. 6a). It also illustrates the association between grazed rotations and reduced weed abundance, increased weed diversity, and increased wheat yields (Fig. 6b).

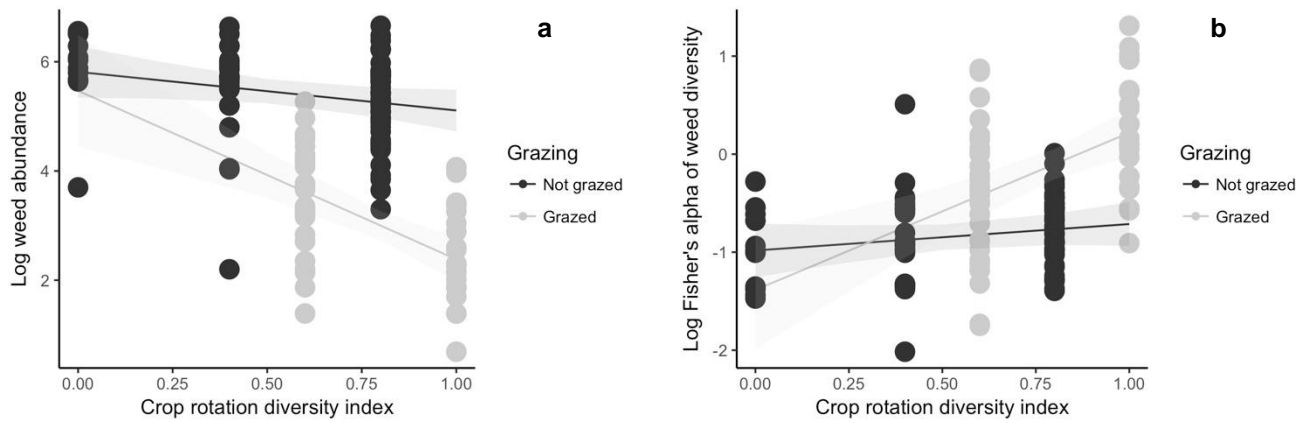


Figure 4: The relationship between weed abundance (a) and weed diversity (b), for grazed (dark shading) and non-grazed (light shading) rotation systems. The lines and ribbons indicate the regression coefficient and 95% confidence interval. This illustrates the interaction between grazing and crop diversity: the effect of crop diversity is greater in grazed than ungrazed systems.

Table 4: The results of the linear mixed models for each index of filter strength or diversity (Table 2).

Results for weed abundance are shown in the left column and for weed diversity in the right, with values given for the fixed effect estimates (random effects not shown) and the P-values calculated by parametric bootstrapping. The Akaike Information Criteria (AIC) is given to compare the goodness-of-fit of models for the different indices, and should be compared amongst abundance models and amongst diversity models, not between the two.

	Abundance		Diversity	
	Estimate	P-value	Estimate	P-value
Model: herbicide AI (g ha⁻¹)				
Herbicide total AI (g ha ⁻¹) index	0.40	0.676	-0.53	0.335
Crop diversity	-5.37	<0.001	1.72	0.001
Interaction (crop div x HX ha ⁻¹)	6.01	<0.001	-1.86	0.019
Period 2	-0.13	0.006	-0.06	0.019
Period 3	0.33		-0.13	
	<i>AIC</i>	396.9	222.2	
Model: grazing				
Grazed	0.17	0.548	0.2	0.228
Crop diversity	-1.9	<0.001	0.94	<0.001
Interactions (crop div x sheep)	1.19	0.003	-0.67	0.005
Period 2	-0.27	0.005	0	0.916
Period 3	0.08		-0.02	
	<i>AIC</i>	353.6	217.2	
Model: N fertiliser (kg ha⁻¹)				
N fertiliser (kg ha ⁻¹) index	0.06	0.949	0.32	0.610
Crop diversity	-5.51	<0.001	2.05	<0.001
Interaction (crop div x N fertiliser)	8.6	<0.001	-2.88	0.004
Period 2	0.01	0.002	-0.08	0.058
Period 3	0.34		-0.09	
	<i>AIC</i>	373.4	226.4	
Model: N source diversity				
N source index	0.31	0.756	-0.84	0.120
Crop diversity	4.38	<0.001	-1.53	0.007
Interaction (crop div x N sources)	-7.35	<0.001	3.01	<0.001
Period 2	-0.27	0.006	0	0.910
Period 3	0.08		-0.02	
	<i>AIC</i>	369.1	221.9	

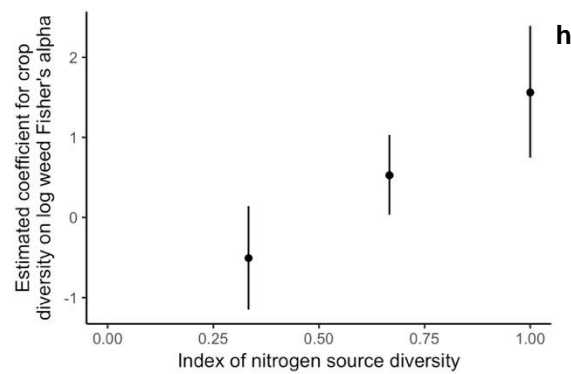
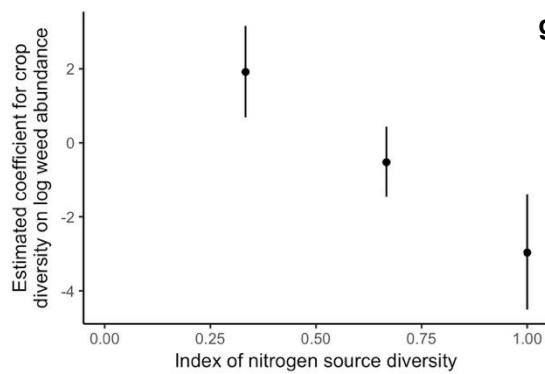
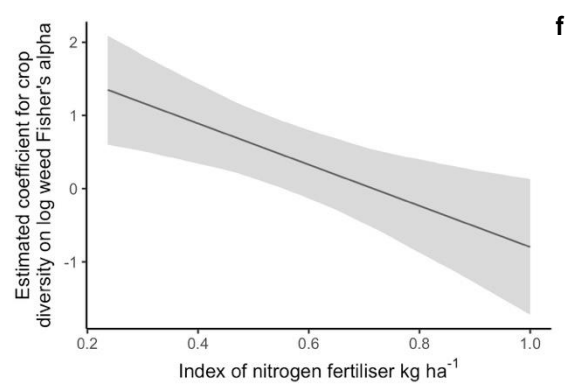
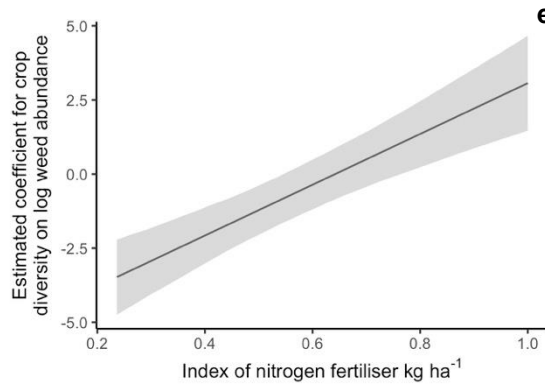
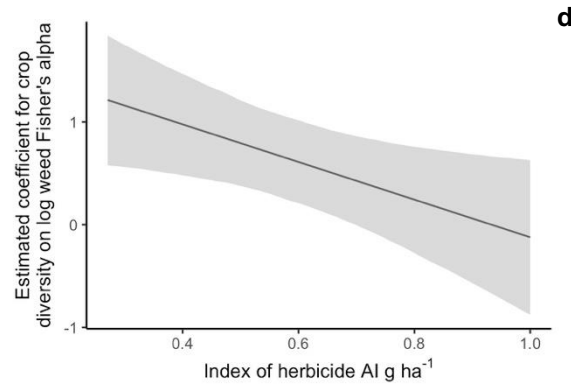
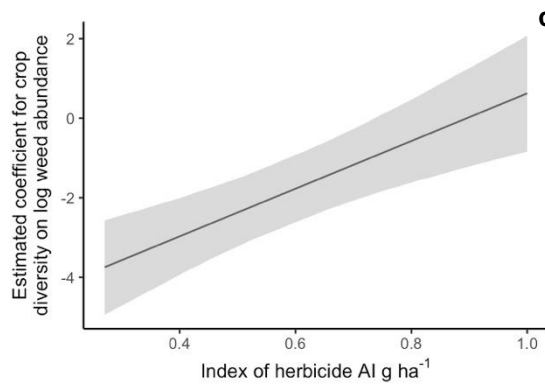
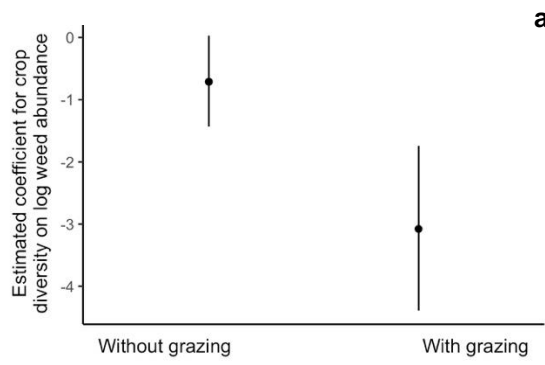


Figure 5: Interaction plots showing the change in the effect of crop diversity on weed abundance and diversity between grazed and ungrazed systems (a and b), as the amount of herbicide applied increased (c and d), nitrogen fertiliser applied increase (e and f) and the diversity of nitrogen sources increased (g and h). These interactions result in the relationships between crop diversity and weeds illustrated in Figure 4: grazed systems had lower amounts of herbicide applied, less fertiliser applied and higher nitrogen source diversity than non-grazed systems (Fig. 2, Table 4).

Table 5: Species observed to emerge in the seedbank samples from the Langgewens Long-Term Trial over the 12 years from 2005 to 2016, and the average proportion of abundance of each species across all plots in all periods of the trial. *Lolium* spp. could not be identified to species level due to hybridisation.

Code	Latin name	Afrikaans name	English name	Status	Average proportion of abundance across all plots in all periods
acal	<i>Arctotheca calendula</i>	gousblom	capeweed	native	<0.01
bdia	<i>Bromus diandrus</i>	predikantluis	ripgut brome	alien	<0.01
calb	<i>Chenopodium album</i>	wit hondebossie	fat hen	alien	<0.01
cot	<i>Cotula</i> spp.	gansogjie	goose-eyes	both	0.04
eaus	<i>Emex australis</i>	dubbeltjie	devil's thorn	alien	<0.01
emos	<i>Erodium moschatum</i>	turknaal	musk heron's bill	alien	0.04
lol	<i>Lolium</i> spp.	raaigras	ryegrass	alien	0.77
mpar	<i>Malva parviflora</i>	kiesieblaar	mallow	alien	0.03
pavi	<i>Polygonum aviculare</i>	litjiesgras	knotweed	alien	0.09
rrap	<i>Raphanus raphanistrum</i>	ramenas	wild radish	alien	<0.01

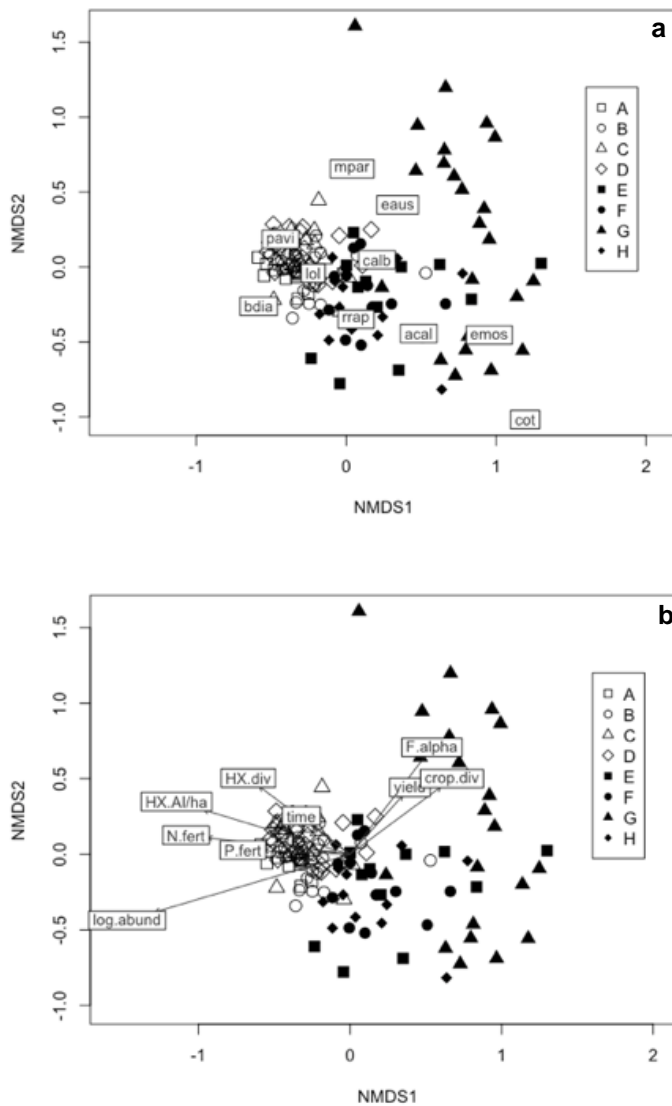


Figure 6: Two-dimensional non-metric multidimensional scaling ordination of the weed communities of each plot in each period. Symbols indicate plots belonging to the different rotation systems; shaded symbols are grazed systems. Labels on (a) represent the species associated with samples in different parts of the ordination, based on weighted averages (see Table 5 for species abbreviations). Arrows on figure (b) represent significant correlations ($P < 0.05$) between variation in community composition and management and resource indices (Table 2), as well as weed abundance (“log.abund”), Fisher’s alpha diversity (“F.alpha”) and wheat yield (“yield”). The length of the arrows is relative to the strength of the correlation. Time is plotted as a continuous variable: change between periods was significant, but the direction of change sufficiently small that plotting periods as category centroids is confusing to the eye.

4. Discussion

In this trial, diverse cropping systems with integrated livestock offered the best outcomes for farm productivity and environmental protection: fewer agrichemicals were applied, weed abundance was lower, weed diversity was higher, and wheat yields were higher. The greatest differences in weed management were between grazed systems (E-H) and ungrazed systems (A-D), but crop sequence diversity also contributed. Overall, the most diverse grazed system (G) performed best, while the wheat monoculture (system A) performed worst. The results of the models of management and resource indices further support that grazing and crop diversity are the strongest drivers of weed abundance and diversity within this trial (Table 4, Fig. 4, Fig. 5).

The strong impact of grazing on weeds supports our first hypothesis that the diversity of management filters, in particular disturbance diversity, can suppress weed abundance and promote weed diversity. Introducing a grazed phase to a rotation adds a disturbance with a distinct selection pressure from herbicides, which may explain why grazing was found to have an impact on weed abundance and diversity, but herbicide group diversity was not (Table 4). Although different herbicides target different species, all herbicides would impose selection pressure for traits that permit general herbicide tolerance or avoidance (such as lower leaf permeability, variable germination times or early maturity; Gaba et al 2017). In contrast, grazing selects for traits that confer unpalatability or resilience to physical defoliation. This suggests that maximising differences in selection pressure between management filters results in more effective weed management.

Findings from other studies on integrated crop-livestock systems support this conclusion that it is filter diversity, rather than grazing in itself, that offers the greatest benefits for weed management. For example, Miller et al (2015) found that replacing herbicide-based or tillage-based management of a forage crop with grazing did not consistently improve weed

suppression, while Lehnhoff et al (2017) show that grazing can reduce reliance on tillage in organic systems but not completely eliminate the need for it. Thus, it is combining distinct selection pressures that is most effective to suppress weeds. Where integrating forage crops and livestock is not practical for farmers, incorporating a mown cover crop may have similar benefits through exerting a similar filter on weeds (McKenzie et al 2016), except in cases where problematic weed species are particularly susceptible to grazing (Leon and Wright 2018).

In this study, the greater dominance of *Lolium* spp. and *P. aviculare* in crop-only systems (Fig. 6) illustrates the effect of the contrasting selection pressures between herbicides and grazing, and the specific effects of grazing on susceptible weeds. Both weeds possess traits conferring herbicide tolerance but both are palatable to sheep. As such, *Lolium* spp. and *P. aviculare* could be viewed as additional forage species promoted by cash crop phases, and the grazing phases as an important strategy for managing these weeds, particularly for any herbicide resistant populations. Resistant *Lolium* is a widespread problem in the Western Cape, and although it is not known whether *Lolium* present in the trial was resistant, this could explain the dramatic differences in weed abundance between the grazed and ungrazed systems. It remains uncertain whether mowing would have similar benefits in these systems.

Previous long-term studies that included two weed control measures with different selection pressures, such as herbicides and tillage, have often not observed reductions in weed biomass when compared with chemical control only (e.g. Chikowo et al 2009, Benaragama et al 2016). However, in such studies, both management actions are typically applied in every year, regardless of crop type. This would create a stronger filter for weed species that can tolerate both management actions, rather than enhancing filter diversity by selecting for tillage-tolerant weeds in one year and herbicide-tolerant weeds in another. In contrast, trials involving more inter-annual variation in management appear to achieve better weed

outcomes (Blackshaw et al 2008, Davis et al 2012, Anderson 2015). In this study, management in the grazed systems varied between high herbicide use in cash crop years, and low herbicide use with grazing in forage crop years. Varying selection pressures between years may therefore be key to successful integrated weed management, although this has yet to be explicitly tested.

In addition to higher filter diversity, the grazed systems in this study also had less herbicide applied (lower disturbance strength), less nitrogen fertiliser applied (lower maximum resource availability), and a higher nitrogen source diversity than ungrazed systems (Fig. 2). From an applied perspective, this provides evidence that integrating livestock permits weeds to be suppressed and yields to be maintained at lower levels of agrichemical inputs, offering both environmental and economic benefits (Petit et al 2015, Basson 2017). However, from a theoretical perspective, this collinearity makes it difficult to distinguish the relative roles of the mechanisms identified in our hypotheses: the effect of disturbance diversity induced by grazing may have been further enhanced by these other attributes of grazed systems (Storkey et al 2010, Smith et al 2010, Gaba et al 2013, Reich 2014).

The effect of crop diversity on weed abundance and diversity in this study was smaller than that of grazing (Fig. 3, Fig. 4), but still important, given that other management variables were significant only in interaction with crop diversity (Table 4, Fig. 5). Crop diversity could affect the weed community through variation in filters imposed by competition, as different crops compete more strongly with certain weeds than others (Petit et al 2015, Nichols et al 2015). Differences in the timing of crop sowing often play a role in determining which weed species emerge, but in this trial all crops were sown at the same time. Several other studies on weed responses to crop rotation have found little or no effect of crop diversity independent of management diversity (Smith and Gross 2007), but the functional differences between the crops in this study were relatively large, and thus may have had a greater effect on weeds.

In contrast to weed abundance and diversity, average wheat yields were more strongly related to crop sequence diversity than to grazing, and were highest in the four most diverse systems (C, D, G and H). Crop diversity contributes to yield in several ways, for example through increasing soil nutrient content and reducing disease, and such effects may be more important to yield than weed suppression (Davis et al 2012, Benaragama et al 2016). The main advantage of integrating livestock into rotation systems is thus not necessarily to improve crop yields, but to decrease the amount of herbicide required for satisfactory weed management. A separate study investigating the economics of the rotation systems in the Langgewens Long-Term Trial found that although yields were comparable between diverse grazed and ungrazed systems, the reduced cost of inputs and increased diversity of marketable outputs in grazed rotations resulted in higher long-term farm profits (Basson 2017).

This study emphasises the benefits that diverse cropping systems with integrated livestock can offer to farmers, agroecosystems, and the natural environment (Davis et al 2012, Sanderson et al 2015). Forage crops provide an opportunity to increase crop diversity, which benefits cash crop yields and reduces fertiliser requirements, while the grazing action of livestock improves weed management and facilitates reductions in herbicide use. Integrating livestock forage phases may therefore prove valuable to sustain arable crop production in the face of herbicide resistance, and to reduce the risks associated with intensive agrichemical use, particularly in no-till systems where non-chemical weed management options are limited. Likewise, there may be potential to improve weed management in organic and low-input cropping systems using grazed forage phases to provide an additional filter alongside mechanical weeding and tillage. Future research could focus on how management actions that apply different selection pressures to weeds can best be integrated in rotation systems, to allow farmers to optimise the use of the weed management tools that are available to them.

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Author contributions

J. Strauss manages the Langgewens Long-Term Trial and oversaw all data collection for this study. C.M., J. Storkey and K.D. planned how to analyse the data to answer the questions posed in this article, and C.M. undertook the data analysis with support from J. Storkey. CM drafted the manuscript with advice from P.S. and K.D. All authors contributed critical revisions to the manuscript.

Data accessibility

The data used in this article has been uploaded with the DOI 10.5281/zenodo.1308220, and can be accessed through www.zenodo.org.

References

Anderson, R.L., 2015. Integrating a complex rotation with no-till improves weed management in organic farming. A review. *Agron. Sustain. Dev.* 35, 967–974.

- Ball, D. and Miller, S. 1989. A Comparison of Techniques for Estimation of Arable Soil Seedbanks and Their Relationship to Weed Flora. *Weed Res.* 29 (5), 365–373
- Basson, C. 2017. A financial analysis of different livestock management approaches within different crop rotation systems in the Middle Swartland. Masters thesis, Stellenbosch University.
- Benaragama, D., Shirliffe, S.J., Gossen, B.D., Brandt, S.A., Lemke, R., Johnson, E.N., Zentner, R.P., ... Moulin, A., Stevenson, C., 2016. Long-term weed dynamics and crop yields under diverse crop rotations in organic and conventional cropping systems in the Canadian prairies. *F. Crop. Res.* 196, 357–367.
- Blackshaw, R.E., Pearson, D.C., Larney, F.J., Regitnig, P.J., Nitschelm, J.J., and Lupwayi, N.Z. (2015) 'Conservation Management and Crop Rotation Effects on Weed Populations in a 12-Year Irrigated Study'. *Weed Tech.* 29, 835–843
- Booth, B.D., Swanton, C.J., 2002. Assembly theory applied to weed communities. *Weed Sci.* 50, 2–13.
- Chikowo, R., Faloya, V., Petit, S., Munier-Jolain, N.M., 2009. Integrated Weed Management systems allow reduced reliance on herbicides and long-term weed control. *Agric. Ecosyst. Environ.* 132, 237–242.
- Crews, T.E. and Peoples, M.B. 2005. Can the Synchrony of Nitrogen Supply and Crop Demand Be Improved in Legume and Fertilizer-Based Agroecosystems? A Review. *Nutr. Cycl. Agroecosys.* 72 (2), 101–120
- Davis, A.S., Hill, J.D., Chase, C.A., Johanns, A.M., Liebman, M., 2012. Increasing Cropping System Diversity Balances Productivity, Profitability and Environmental Health. *PLoS One* 7.
- Ferreira, M. I., Reinhardt, C. F., Lamprecht, S. C., Sinclair, M., MacKenzie, L., van Coller, G. 2015. Morphological identification of the ryegrass hybrid *Lolium multiflorum* × *Lolium perenne* and isolation of the pathogen *Fusarium pseudograminearum* in the Western Cape. *S. Afr. J. Plant and Soil* 32(1), 9-15.

- Gaba, S., Perronne, R., Fried, G., Gardarin, A., Bretagnolle, F., Biju-Duval, L., Reboud, X., 2017. Response and effect traits of arable weeds in agro-ecosystems: a review of current knowledge. *Weed Res.* 57, 123–147.
- Gaba, S., Gabriel, E., Chadœuf, J., Bonneu, F., Bretagnolle, V., 2016. Herbicides do not ensure for higher wheat yield, but eliminate rare plant species. *Sci. Rep.* 6, 30112.
- Gaba, S., Fried, G., Kazakou, E., Chauvel, B., Navas, M.L., 2013. Agroecological weed control using a functional approach: A review of cropping systems diversity. *Agron. Sustain. Dev.* 34, 103–119.
- Gross, K. (1990) 'A Comparison of Methods for Estimating Seed Numbers in the Soil'. *J. Ecol.* 78 (4), 1079–1093
- Halekoh, U., Højsgaard, S., 2014. A Kenward-Roger Approximation and Parametric Bootstrap Methods for Tests in Linear Mixed Models. *J. Stat. Softw.* 59.
- Isbell, F., Adler, P.R., Eisenhauer, N., Fornara, D., Kimmel, K., Kremen, C., ... Scherer-Lorenzen, M., 2017. Benefits of increasing plant diversity in sustainable agroecosystems. *J. Ecol.* 105, 871–879.
- Lehnhoff, E., Miller, Z., Miller, P., Johnson, S., Scott, T., Hatfield, P., and Menalled, F.D. 2017 Organic Agriculture and the Quest for the Holy Grail in Water-Limited Ecosystems : Managing Weeds and Reducing Tillage Intensity. *Agriculture* 7 (33)
- Leon, R.G. and Wright, D.L. 2018. Recurrent Changes of Weed Seed Bank Density and Diversity in Crop—Livestock Systems. *Agron. J.* 110(3), 1068-1078
- Magurran, A.E. (2003) *Measuring Biological Diversity*. Blackwell, Oxford.
- McKenzie, S.C., Goosey, H.B., O'Neill, K.M., and Menalled, F.D. 2016. Impact of Integrated Sheep Grazing for Cover Crop Termination on Weed and Ground Beetle (Coleoptera:Carabidae) Communities. *Agric. Ecosyst. Environ.* 218, 141–149

- Miller, Z.J., Menalled, F.D., Lenssen, A.W., and Patrick, G. 2015. Integrating Sheep Grazing into Cereal-Based Crop Rotations: Spring Wheat Yields and Weed Communities. *Agron. J.* 107, 104–112
- Mortensen, D.A., Egan, J.F., Maxwell, B.D., Ryan, M.R., Smith, R.G., 2012. Navigating a Critical Juncture for Sustainable Weed Management. *Bioscience* 62, 75–84.
- Navas, M.L., 2012. Trait-based approaches to unravelling the assembly of weed communities and their impact on agro-ecosystem functioning. *Weed Res.* 52, 479–488.
- Neve, P., Vila-Aiub, M. Roux, F. (2009) Evolutionary-thinking in agricultural weed management. *New Phytol.* 184(4), 783-93.
- Nichols, V., Verhulst, N., Cox, R., Govaerts, B., 2015. Weed dynamics and conservation agriculture principles: A review. *F. Crop. Res.* 183, 56–68.
- Paul, E., Keith, W.T., James, R., Peter, R., 1998. Long-term agroecosystem experiments: Assessing agricultural sustainability and global change. *Science* 282, 893-896.
- Petit, S., Munier-Jolain, N., Bretagnolle, V., Bockstaller, C., Gaba, S., Cordeau, S., Colbach, N., 2015. Ecological intensification through pesticide reduction: weed control, weed biodiversity and sustainability in arable farming. *Environ. Manage.* 56, 1078–1090.
- Poudel, D.D., Horwath, W.R., Lanini, W.T., Temple, S.R., Bruggen, A.H.C. Van, 2002. Comparison of soil N availability and leaching potential, crop yields and weeds in organic, low-input and conventional farming systems in northern California. *Agric. Ecosyst. Environ.* 90, 125–137.
- Pretty, J. Bharucha, Z.P. (2014) Sustainable intensification in agricultural systems. *Ann. Bot.* 114(8), 1571-96.
- R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: <https://www.R-project.org/>.

- Reich, P.B., 2014. The world-wide “fast–slow” plant economics spectrum: a traits manifesto. *J. Ecol.* 102, 275–301.
- Sanderson, M. A., Archer, D., Hendrickson, J., Kronberg, S., Liebig, M., Nichols, K., ... Aguilar, J., 2013. Diversification and ecosystem services for conservation agriculture: Outcomes from pastures and integrated crop–livestock systems. *Renew. Agric. Food Syst.* 28, 129–144.
- Smith, R.G., Mortensen, D.A., Ryan, M.R., 2010. A new hypothesis for the functional role of diversity in mediating resource pools and weed-crop competition in agroecosystems. *Weed Res.* 50, 37–48.
- Smith, R.G., Gross, K.L., 2007. Assembly of weed communities along a crop diversity gradient. *J. Appl. Ecol.* 44, 1046–1056.
- St-Martin, A., Vico, G., Bergkvist, G., Bommarco, R., 2017. Diverse cropping systems enhanced yield but did not improve yield stability in a 52-year long experiment. *Agric. Ecosyst. Environ.* 247, 337–342.
- Storkey, J., Moss, S.R., Cussans, J.W., 2010. Using assembly theory to explain changes in a weed flora in response to agricultural intensification. *Weed Sci.* 58, 39–46.
- Storkey, J., Meyer, S., Still, K.S., Leuschner, C., 2012. The impact of agricultural intensification and land-use change on the European arable flora. *Proc. R. Soc. B Biol. Sci.* 279, 1421–1429.
- Storkey, J., Macdonald, A.J., Bell, J.R., Clark, I.M., Gregory, A.S., Hawkins, ... Whitmore, A.P. 2016. The Unique Contribution of Rothamsted to Ecological Research at Large Temporal Scales. In *Advances in Ecological Research, Vol 55: Large-Scale Ecology: Model Systems to Global Perspectives* (eds A.J. Dumbrell, R.L. Kordas G. Woodward), pp. 3-42. Elsevier Academic Press Inc.
- Wezel, A., Casagrande, M., Celette, F., Vian, J.F., Ferrer, A., Peigné, J., 2014. Agroecological practices for sustainable agriculture. A review. *Agron. Sustain. Dev.* 34, 1–20.

