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Intercropping Winter Lupin and Triticale Increases Weed Suppression and Total Yield

Nicolas Carton, Christophe Naudin, Guillaume Piva and Guénaëlle Corre-Hellou *

USC LEVA, INRA, Ecole Supérieure d'Agricultures, Univ. Bretagne Loire, SFR 4207 QUASAV 1, 55 rue Rabelais, 49007 Angers CEDEX, France; nicolas.carton1@gmail.com (N.C.); c.naudin@groupe-esa.com (C.N.); g.piva@groupe-esa.com (G.P.)

* Correspondence: g.hellou@groupe-esa.com

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Abstract: Lupin (*Lupinus* sp.) produces protein-rich grains, but its adoption in cropping systems suffers from both its low competitive ability against weeds and its high yield variability. Compared with legume sole cropping, grain legume–cereal intercropping benefits include better weed suppression and higher yield and yield stability. However, the potential of enhancing crop competitive ability against weeds in additive winter grain legume–cereal intercrops is not well-known, and this potential in long crop cycles is even less studied. We studied how intercropping with a triticale (×*Triticosecale*) alters weed biomass and productivity of winter white lupin (*Lupinus albus* L.). The experimental setup consisted of eleven sites during a two-year period in western France. In each site-year, winter white lupin sole cropping was compared to winter white lupin-triticale intercropping in an additive sowing design. We found that intercropping reduced weed biomass at lupin flowering by an average of 63%. The rapid growth and high soil N acquisition of triticale compensated for the low competitive ability of lupin against weeds until lupin flowering. Competition from triticale in the intercrop reduced lupin grain yield (−34%), but intercropping produced a higher total grain yield (+37%) than did lupin sole cropping while maintaining the total protein grain yield.

Keywords: intercropping; lupin; triticale; weeds; legumes; nitrogen

1. Introduction

In Europe, the livestock sector mostly relies on imported soybean cake as protein-rich feed [1]. Local protein-rich crop products are needed to increase self-sufficiency. Among candidate crops, lupins (*Lupinus albus* (white lupin), *L. angustifolius* (narrow-leafed lupin)) produce seeds that have the highest grain protein content (30–42%) among grain legumes, and these seeds can partly substitute for soybean in ruminant, pig and poultry diets [2]. Lupins can also be an alternative to soybean in food diets including more plant proteins [3]. Lupins also fix significant amounts of atmospheric nitrogen (N_2) with an average fixation rate of 75% [4]. Like other grain legumes, lupins can provide farming systems with additional services by contributing to crop diversification and reducing synthetic nitrogen (N) fertilizer requirement in crop rotations. However, lupins are not widely cultivated in the European Union (EU) (approximately 120,000 ha in 2014; [5]) because of the high yield variability of this crop [6]. This variability is presumably associated with its high susceptibility to biotic and abiotic stresses. High weed infestation levels are usually reported [7,8]; these infestations are likely due to the slow ground cover and the long cropping season of the crop, especially for winter white lupin.

Compared with sole cropping, intercropping of grain legumes with cereals is a cropping strategy that can increase yield and improve yield stability, especially under low-input conditions [9–11]. This phenomenon generally results from the improved use of abiotic resources (light interception

and use of both soil mineral N and atmospheric N_2). Intercropping can also reduce insect pests [12], diseases [13] and weeds [14,15].

Farmers often cite weeds as the main challenge in grain legumes. Intercropping a grain legume with a cereal can reduce weed growth. For instance, in spring pea, intercrops with barley suppress more weed biomass than do sole pea crops [15,16].

While pea– and faba bean–cereal intercropping has been the subject of numerous studies, white lupin–cereal intercropping for grain harvest is an innovative practice that has received little attention from academic researchers. Among grain legumes, lupin exhibits both the most variable yield [6] and the least competitive ability against weeds [7,17], meaning that intercropping winter white lupin for grain may have a high potential of development.

The objective of the practice described in this study is to produce protein-rich grains and the originality is that lupin is the main crop and the cereal is a companion crop that is also expected to produce grain ("harvested companion crop"). Intercropping lupin with a cereal could promote lupin cropping if the practice can reliably circumvent the two main shortcomings of lupin sole cropping by increasing competitive ability against weeds and securing grain and protein production.

The combination of intercropped species that exhibit contrasting traits could increase both the use of available resources and the competitive ability of the mixture against weeds. Compared with legume sole cropping, intercropping two species supposedly results in a higher competitive ability, especially at the beginning of the crop cycle, due to the contrasting traits of both species. In lupin–cereal intercrops, we expect both lupin early growth and soil N acquisition to be low and cereal growth and N acquisition to compensate for the low early competitive ability of lupin against weeds. In a multisite study in Europe, cereal competitive ability for soil mineral N was decisive regarding the higher weed suppression in organic spring pea–barley intercrops than in sole-cropped pea [16]. Species interactions can vary over time, especially during long cycle crops. To better understand the ultimate performances of legume–cereal intercrops, the systematic description of the relative dominance of each species before the period of maximum growth and maximum N_2 fixation rate of the legume would be useful because the benefits of intercropping for resource use start in the early growth phase [18] and because early dominance can shape the interactions in the second half of the growth cycle.

The aim of the present study was to compare the weed suppression and yield performance between winter white lupin-triticale intercropping and lupin sole cropping. The original aspect of this work is to study the effect of the addition of triticale by analyzing two phases: from sowing to lupin flowering and from lupin flowering to maturity. Moreover, the interest of this study lies in the fact that a range of contrasting growth conditions was used which will help to understand the conditions needed to guarantee the success of this practice. This was achieved by comparing winter white lupin–triticale intercropping and lupin sole cropping throughout a set of eleven experiments during a two-year period in western France.

2. Materials and Methods

2.1. Field Sites

Field experiments were carried out in the 2014/15 and 2015/16 growing seasons in western France for a total of eleven site-years (see details in Table 1). The 20-year average annual rainfall in the area is 718 mm, and average annual air temperature is 12.5 °C. The weather patterns of the two study years deviated similarly from the 20-year average. Specifically, the main deviation from the average data involved the October-February air temperatures, which were 8.9 °C (2014/15) and 9.6 °C (2015/16) averaged over the study sites, whereas the 20-year average was 8.2 °C (Figure 1). At each site, two winter white lupin cropping strategies were compared: lupin sole cropping and lupin–winter triticale intercropping.

Table 1. Site details of the experimental fields.

Year	2015						2016				
Site	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**
Location	47.40 N, 1.32 W	47.87 N, 0.20 E	47.51 N, 1.48 W	47.06 N, 1.31 W	47.46 N, 1.23 W	47.64 N, 1.51 W	47.53 N, 1.03 W	47.47 N, 0.40 W	47.39 N, 1.33 W	47.51 N, 1.48 W	47.78 N, 1.45 W
Experimental design	strips	blocks	strips	blocks	strips	strips	strips	blocks	strips	strips	blocks
Soil texture	Loam	sand	silt loam	sandy loam	loam	silt loam	loam	sand	loam	silt loam	silt loam
Sowing date (day/month)	27/09	25/09	13/09	26/09	5/10	2/10	30/09	1/10	25/09	23/09	29/09
Varieties: Lupin Triticale	Lumen Ragtac	Clovis Kaulos	Clovis Ragtac	Clovis Ragtac	Lumen Ragtac	Lumen Ragtac	Lumen -Clovis (50% mix) Ragtac	Magnus Vuka	Orus Ragtac	Lumen Ragtac	Magnus Ruminac
Sowing density (kernels·m^{-2}): Lupin Triticale	 28 75	 25 75	 30 70	 25 70	 27 72	 31 70	 25 87	 30 75	 30 60	 25 75	 25 75
Triticale sowing	close to lupin rows	same rows	same rows	alternating rows	alternating rows	alternating rows	same rows	alternating rows	close to lupin rows	same rows	alternating rows
Preceding crop	winter wheat	winter wheat	winter wheat	winter barley	winter wheat	winter wheat	winter wheat	rapeseed	winter wheat	winter wheat	forage maize
Weed control C: chemical M: mechanical (number of operations)	C (1) M (1)	C (1)	C (1)	C (2)	C (2)	C (1)	C (1)	0	C (1)	C (1)	C (2)
Lupin plant density after winter in the sole crop (pl·m^2)	17	n.d.	40	n.d.	27	24	14	29	26	22	n.d.
Lupin plant density after winter in the intercrop (pl·m^2)	20	n.d.	21	n.d.	24	41	14	26	24	17	n.d.
Available N at sowing (0–90 cm) (kg·ha^{-1})	81	n.d.	119	n.d.	67	206	121	95	104	124	n.d.

n.d.: not determined.

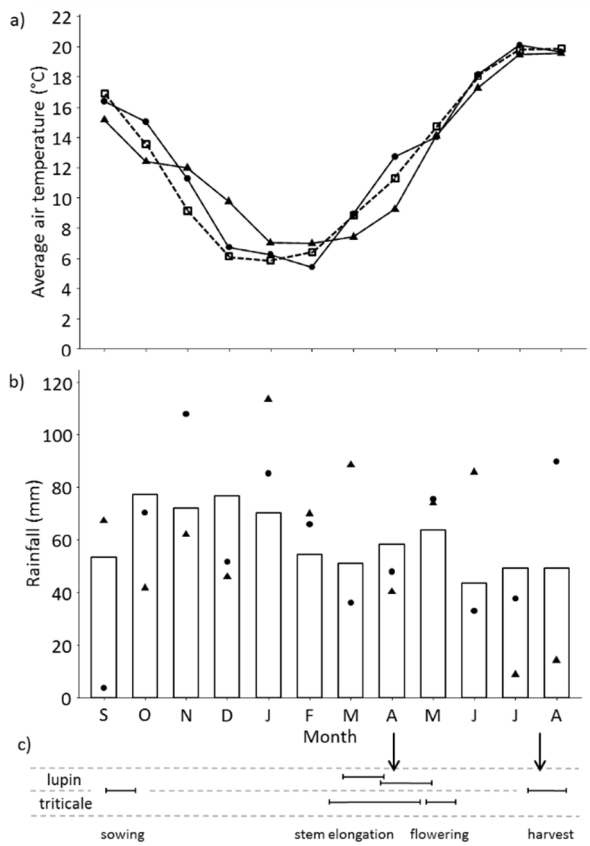

Figure 1. (**a**) Monthly average air temperature, (**b**) monthly rainfall, averaged over the study sites in 2015 (dots) and 2016 (triangles) as well as the 20-year average (temperature: open squares, rainfall: bars) and (**c**) average timing of key events of the crop cycle. Arrows represent the two harvests: at lupin main stem flowering and at lupin maturity.

The eleven sites were seven lupin-triticale intercropped farm fields (minimum 1 ha) on real farms including a wide strip (minimum 10 × 100 m) of sole-cropped lupin and four microplot experiments in a randomized block design with four replicates, including sole-cropped winter white lupin and lupin-triticale intercrop (individual plots ranging from 3 × 10 m to 6 × 17 m). The interest of these two sources of data was to involve different actors (farmers, advisers, researchers) and investigate the effect of intercropping in a wide range of situations. All fields were managed with conventional farming practices. Decisions of agricultural practices including cultivars; sowing date; preceding crop; row width; intercrop spatial arrangement; and pest, disease and weed control were made by both farmers and experiment managers and varied among sites (Table 1). However, the sole-cropped lupin and intercrop were managed identically, with the exception of the site F, in which the intercrop seedbed preparation included less soil tillage than did the lupin sole crop and 50 kg N·ha^{-1} was added only to the intercrop in April. The other sites were managed without N fertilization both in sole and intercrops. Lupin row spacing ranged between 12 and 75 cm (average: 34 cm). In all intercrops, the two species were sown on the same day or within one day. The lupin cultivars were chosen from the dwarf determinate branched cultivars that are typically cultivated in western France. Triticale cultivars were chosen for their late maturity, which is desirable for the simultaneous maturity of both species in the intercrop. Tested lupin cultivars do not show major differences except for the greater plant height of cv. Magnus. Tested triticale cultivars do not show major differences; all have a medium plant height. The lupin seeds were inoculated with *Bradyrhizobium lupini* in accordance with commonly recommended practices. The sole-cropped lupin was sown at an average of 27 kernels·m^{-2} (Table 1; SD = 2.4 kernels·m^{-2}). Lupin in the lupin-triticale intercrop was sown at the same density as lupin in the sole crop; the average triticale sowing density was 73 kernels·m^{-2} (SD = 6.1 m^{-2}), corresponding to

an additive design in which lupin (L) was sown at the recommended density, and triticale (T) was sown at 30% of the sole crop recommended sowing density (L100:T30). All sites were rain fed and received no supplemental irrigation. All sites except H received a chemical control with only one application before emergence for sites A, B, C, D, F, G, I, J and with two applications, before emergence and during the winter for sites E and K. One site (A) also received a mechanical weeding in spring. These chemical or mechanical operations were similar in intercrops and sole crops.

2.2. Measurements, Sampling and Analysis

At all sites, the aboveground parts of lupin, triticale and weeds were hand-harvested twice throughout the crop cycle: at lupin main-stem flowering (April) and at lupin maturity (July until beginning of August). In the seven real farms, six plots (20 × 30 m) were defined randomly: three in the intercropped strips and three in the sole-cropped lupin strips. In each plot, the plants were harvested in three randomly defined subplots that covered 1 m × 2 lupin or lupin + triticale rows, and the values were averaged across subplots. In the four randomized block design experiments, plot size ranged between 1.7 × 10 m to 4.5 × 20 m and the plants were harvested in each plot in a randomly defined subplot (minimum area: 0.3 m^2; maximum area: 1.05 m^2). In the same plots later used for biomass sampling, all weeds were identified. The aboveground dry matter (DM) was determined after oven drying at 70 °C for 48 h until constant weight. At harvest, the grain and straw were threshed and then weighed. For N content measurements on aboveground biomass of lupin and triticale at lupin flowering, and on lupin and triticale grain and straw at maturity and aboveground weed biomass at flowering and maturity, the samples were pooled each across blocks and ground (120-mm mesh netting; "Pulverisette 19" universal cutting mill, "Laborette 27" sampler, and "Pulverisette 14" variable speed rotor mill; Fritsch, Idaroberstein, Germany). The total N concentration and ^{15}N:^{14}N ratio measurements were performed using a CHN analyser (EA3000; Euro Vector, Milan, Italy) and a mass spectrometer (IsoPrime; Elementar, Hanau, Germany). The mineral soil N content of representative soil samples from a 0–90-cm depth at sowing was measured via segmented flow analysis (Skalar Analytical B.V., Breda, Netherlands), which enables the determination of nitrate and ammonium contents by extraction with KCl [19]. At eight sites (A, C, E, F, G, H, I, J), crop plant density after emergence and lupin density after winter were recorded, and he mineral soil N content was measured after winter (0–90 cm). Protein content was determined by N content multiplied by 6.25.

2.3. Calculations

Weed reduction (WR) was assessed to characterize the ability of the intercrop to suppress weeds compared to the lupin sole crop. The index was determined according to the following equation:

$$\text{WR} = 100 \times ((\text{weed DM in the lupin sole crop} - \text{weed DM in the intercrop})/(\text{weed DM in the lupin sple crop})) \quad (1)$$

The percentage of accumulated N derived from the air (%Ndfa) in lupin was determined on the two sampling dates using the ^{15}N natural abundance method [20]. Triticale served as the non-fixing reference in the calculation. The following equation was used:

$$\%\text{Ndfa} = 100 \times ((\delta^{15}N_{legume} - \delta^{15}N_{reference}) - (\beta\text{fix} - \delta^{15}N_{reference})) \quad (2)$$

where $\delta^{15}N_{legume}$ and $\delta^{15}N_{reference}$ are the natural ^{15}N enrichment values of the legume and triticale, respectively. The β-values for lupin ("βfix") were derived from the minimum values attained by $\delta^{15}N$ at all the sites: −0.88 at flowering (site H) and −1.03 at maturity (site D).

The normality and homoscedasticity of model residuals were tested using Shapiro's and Levene's tests, respectively (α = 0.05). For the across-sites statistics, per site means of the data were used and the differences between sole-cropped lupin and the intercrop were assessed by Student's paired *T*-tests (α = 0.05) except when non-normality was detected for model residuals. In those cases, Wilcoxon's

signed-rank test ($\alpha = 0.05$) was used. Linear regressions using model II (Reduced Major Axis) were computed to assess relationships between variables. The absence of outliers in the data was assessed with Grubbs' test of model residuals [21]. For the per site statistics, per block data were used and the differences between sole-cropped lupin and the intercrop on each site were assessed by Student's *T*-tests ($\alpha = 0.05$), using the pooled variance estimate calculated using all sites. The Benjamini and Hochberg method was used to control the false discovery rate, i.e., the expected proportion of false discoveries amongst the rejected hypotheses [22]. Individual per site *T*-tests were used for lupin grain yield and crop total grain yield, where the global model was not applicable because of variance heterogeneity. All statistical analyses were performed using R software [23] version 3.3.2.

3. Results

3.1. Weed Suppression

The treatments were compared under various situations of weed infestation and growing conditions. In the lupin sole crop, at maturity, weed biomass ranged from 0 g·m^{-2} (site K) to 567 g·m^{-2} (site G; Table 2). The sites differed also in weed communities (Table 3).

Crop biomass of the different treatments at maturity also varied widely across sites, from 109 g·m^{-2} to 1238 g·m^{-2} in the lupin sole crop and from 416 g·m^{-2} to 1850 g·m^{-2} in the lupin-triticale intercrop. The variability of crop and weed growth was higher among sites than between the two years; therefore, the year effect was not isolated in the analyses.

The weed biomass at lupin flowering was lower in the intercrop (on average 38 g·m^{-2}; Table 4) than in the lupin sole crop (average of 100 g·m^{-2}).

The weed reduction (WR) reached an average of 63%. The difference in weed biomass between the lupin sole crop and the intercrop was higher with higher levels of weed biomass (Figure 2a).

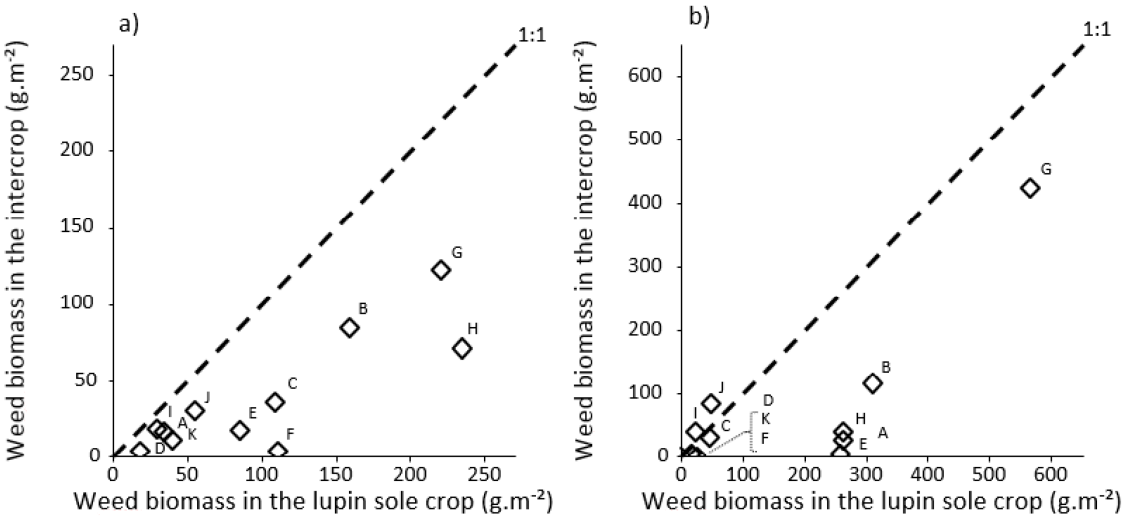

Figure 2. (**a**) Weed biomass in the intercrop against weed biomass in the lupin sole crop at lupin flowering and (**b**) at lupin maturity. Letters next to points identify study sites. The dashed lines represent the theoretical situations with equal weed biomass in both cropping strategies.

Table 2. Weed biomass (dry matter), crop biomass (dry matter), lupin grain dry yield and total grain dry yield in lupin sole crop (SC) and lupin-triticale intercrop (IC) on the 11 sites.

		Weed Biomass (g. m^2)								Crop Biomass at Lupin Flowering (g·m^2)						Lupin Grain Yield (g·m^2)			Total Grain Yield (g·m^2)		
		At Lupin Flowering				at Maturity				Lupin			Crop Total								
Site	Treatment	Mean	SD	Global *t*-test	WR (%)	Mean	SD	Global *t*-test	WR (%)	Mean	SD	Global *t*-test	Mean	SD	Global *t*-test	Mean	SD	Per Site *t*-test	Mean	SD	Per Site *t*-test
A	Lupin SC	34	25	n.s.	53	264	131	***	90	74	33	n.s.	74	33	***	33	21	n.s.	33	21	*
	IC	16	20			26	17			51	5		424	93		53	13		248	39	
B	Lupin SC	160	34	*	47	311	97	***	63	80	53	n.s.	80	53	***	236	117	n.s.	236	117	n.s.
	IC	85	48			117	26			122	56		302	133		315	161		462	163	
C	Lupin SC	109	37	n.s.	67	47	58	n.s.	37	528	58	***	528	59	***	271	80	n.s.	271	80	n.s.
	IC	35	22			30	11			196	57		852	34		118	6		228	111	
D	Lupin SC	19	18	n.s.	81	17	30	n.s.	79	333	129	***	333	129	***	370	82	n.s.	370	82	n.s.
	IC	4	5			4	6			206	88		898	302		227	82		523	62	
E	Lupin SC	86	50	n.s.	79	259	86	***	99	117	40	n.s.	117	40	***	381	102	*	381	102	n.s.
	IC	18	10			3	2			77	17		501	103		123	31		608	30	
F	Lupin SC	111	67	*	97	267	24	n.s.	100	243	75	*** [1]	243	75	***	445	21	*	445	21	n.s.
	IC	3	4			0	0			399	25		1070	126		401	8		628	72	
G	Lupin SC	221	51	*	45	567	90	**	25	59	32	n.s.	59	32	***	75	43	n.s.	75	43	n.s.
	IC	122	47			424	49			47	22		386	13		40	8		68	10	
H	Lupin SC	235	52	***	70	263	75	***	85	154	34	n.s.	154	34	***	218	86	n.s.	218	86	*
	IC	71	42			40	29			111	21		552	167		183	43		399	55	
I	Lupin SC	29	25	n.s.	37	23	28	n.s.	−70	325	47	***	325	47	n.s.	259	9	n.s.	259	9	n.s.
	IC	19	13			40	11			160	19		430	46		208	54		285	37	
J	Lupin SC	55	26	n.s.	45	48	42	n.s.	−73	355	70	***	355	70	**	301	53	*	301	53	n.s.
	IC	31	20			83	59			113	31		550	62		181	25		278	40	
K	Lupin SC	40	74	n.s.	73	0	0	n.s.	n.a.	445	55	***	445	55	***	663	154	*	663	154	n.s.
	IC	11	5			0	0			300	76		999	112		299	42		621	57	

The significance levels of comparisons between IC and lupin SC were assessed with a *t*-test using the pooled variance estimate calculated using all sites ("global *t*-test") or individual per site *t*-tests where the global model was not applicable. n.s.: not significant, n.a.: not applicable; [1] on site F, lupin biomass at flowering was significantly higher in the intercrop than in the sole crop. ***, **, indicate significant differences among species at $p < 0.001$, $p < 0.05$ respectively.

Table 3. List of weed species (species present with more than one plant per m^2 in at least one third of the subplots at lupin flowering) in lupin sole crops (SC) and intercrops (IC).

Sites	SC/IC	List of Weed Species
A	SC	*Atriplex patula*, *Epilobium tetragonum*, *Polygonum aviculare*
	IC	*Attiplex patula*, *Epilobium tetragonum*
B	SC	*Erodium cicutarium*, *Fallopia convolvulus*, *Juncus bufonius*, *Poa annua*, *Senecio vulgaris*, *Viola arvensis*, *Conyza sumatrensis*
	IC	*Erodium cicutarium*, *Fallopia convolvulus*, *Juncus bufonius*, *Poa annua*, *Senecio vulgaris*, *Viola arvensis*
C	SC	*Hypericum perforatum*, *Poa annua*, *Atriplex patula*, *Epilobium tetragonum*, *Polygonum aviculare*, *Ranunculus sardous*, *Senecio vulgaris*
	IC	*Poa annua*, *Phleum pratense*
D	SC	*Hypericum perforatum*, *Polygonum aviculare*, *Stellaria media*, *Atriplex patula*, *Chenopodium album*, *Conyza sumatrensis*, *Epilobium tetragonum*, *Poa annua*, *Portulaca oleracea*, *Ranunculus sardous*
	IC	*Hypericum perforatum*, *Poa annua*, *Ranunculus sardous*
E	SC	*Juncus bufonius*, *Lysimachia arvensis*, *Epilobium tetragonum*, *Hypericum perforatum*, *Poa annua*, *Ranunculus*
	IC	*Raphanus raphanistrum*, *Epilobium tetragonum*, *Geranium dissectum*, *Ranunculus sardous*, *Senecio vulgaris*
F	SC	*Hypericum perforatum*, *Juncus bufonius*, *Geranium dissectum*, *Epilobium tetragonum*, *Fallopia convolvulus*, *Tripleurospermum inodorum*, *Poa annua*, *Polygonum aviculare*, *Ranunculus sardous*, *Senecio vulgaris*
	IC	*Hypericum perforatum*, *Juncus bufonius*, *Tripleurospermum inodorum*, *Poa annua*, *Polygonum aviculare*, *Ranunculus sardous*
G	SC	*Bromus mollis*, *Tripleurospermum inodorum*, *Poa annua*, *Arabidopsis thaliana*, *Daucus carota*, *Fumaria officinalis*, *Rumex crispus*
	IC	*Dactylis glomerata*, *Tripleurospermum inodorum*, *Poa annua*, *Aphanes arvensis*, *Rumex crispus*
H	SC	*Poa annua*, *Juncus bufonius*, *Senecio vulgare*, *Arabidopsis thaliana*, *Capsella bursa-pastoris*, *Conyza sumatrensis*, *Tripleurospermum inodorum*, *Sonchus asper*
	IC	*Poa annua*, *Juncus bufonius*, *Senecio vulgare*, *Arabidopsis thaliana*, *Capsella bursa-pastoris*, *Tripleurospermum inodorum*
I	SC	*Juncus bufonius*, *Poa annua*, *Polygonum aviculare*, *Stellaria media*, *Veronica hederifolia*
	IC	*Juncus bufonius*, *Poa annua*, *Polygonum aviculare*, *Daucus carota*, *Hypericum perforatum*, *Ranunculus sardous*
J	SC	*Daucus carota*, *Poa annua*
	IC	*Daucus carota*, *Poa trivialis*
K	SC	*Elytrigia repens*
	IC	*Elytrigia repens*

Table 4. Crop and weed biomass production and soil N acquisition at the 11 sites in lupin sole crops (SCs) and lupin–triticale intercrops (ICs) during two periods, from sowing to lupin flowering, and from lupin flowering to maturity.

		From Sowing until Lupin Flowering		From Lupin Flowering until Maturity	
		Crops	**Weeds**	**Crops**	**Weeds**
Biomass production ($g \cdot m^{-2}$)	Lupin SC	247 (162)	100 (76)	526 (285)	66 (138)
	IC total	633 (270)	38 (38)	494 (263)	32 (93)
	Lupin in IC	162 (109)		307 (180)	
	Triticale in IC	471 (181)		187 (201)	
comparisons	Lupin SC-IC total	t_{10} = 6.3 ***	t_{10} = −4.2 **	t_{10} = 0.4 (n.s.)	t_{10} = −1.1 (n.s.)
	Lupin SC - Lupin in IC	t_{10} = −2.1 (n.s.)		t_{10} = −3.3 **	
	Lupin in IC - Triticale in IC	t_{10} = −7.9 ***		t_{10} = 1.4 (n.s.)	
Soil N uptake ($g \cdot m^{-2}$)	Lupin SC	2.5 (1.6)	1.9 (1.0)	6.7 (6.9)	0.4 (2.0)
	IC total	7.0 (3.2)	0.7 (0.6)	3.5 (3.6)	0.5 (1.6)
	Lupin in IC	0.9 (0.9)		3.0 (3.2)	
	Triticale in IC	6.2 (2.7)		0.5 (2.5)	
Comparisons	Lupin SC - IC total	t_{10} = 7.4 ***	t_{10} = − 5.5 ***	V = 6 *	t_{10} = 0.3 (n.s.)
	Lupin SC - Lupin in IC	t_{10} = −4.8 ***		V = 5 **	
	Lupin in IC - Triticale in IC	t_{10} = −7.4 ***		t_{10} = 1.9 (n.s.)	

The significance levels of comparisons were assessed with *T*-tests except where *V*, the test statistic of Wilcoxon's signed-rank test, is given. n.d.: not determined; n.s.: not significant. All values are the means (SDs) of plant aboveground dry matter and soil N uptake, $n = 11$. ***, **, indicate significant differences among species at $p < 0.01$, $p < 0.05$ respectively.

A significant effect of intercrop on weed biomass was observed on four sites (B, F, G, H) (Table 2). These sites had a high weed biomass (higher than 110 $g \cdot m^2$).

The weed biomass at maturity was also significantly lower in the intercrop (on average 70 $g \cdot m^{-2}$) than in the lupin sole crop (on average 166 $g \cdot m^{-2}$). However, the WR was lower (average of 43%) at crop maturity than at flowering. Weed reduction in the intercrop compared to sole-cropped lupin occurred mainly from sowing until lupin flowering, and to a lesser extent from lupin flowering until crop maturity. The variability across sites of WR at maturity (coefficient of variation (CV) of 151%) was higher than WR at flowering. (CV of 30%). The average WR at maturity was only 15% for the six sites that had the lowest weed biomass in the sole crop and weed reduction was not statistically significant for these sites. However, the WR was highly significant at maturity and reached 60% for the five sites on which weed biomass in the sole crop surpassed 200 $g \cdot m^{-2}$ (Table 2).

Triticale produced more biomass than did lupin in the intercrop from sowing until lupin flowering (Table 4). The addition of triticale systematically significantly increased total crop biomass at lupin flowering except on site I (average of +387 $g \cdot m^{-2}$, i.e., +157% DM, $t_{10} = 6.3$, $p = 8\ E^{-5}$, minimum: +32% (site I), maximum: +549% (site G), Figure 3. At lupin flowering, the WR was linearly correlated with the crop total biomass gain allowed by the integration of triticale (Figure 4).

Intercropping allowed a median crop biomass of 551 $g \cdot m^{-2}$, and the weed biomass was maintained at less than 50 $g \cdot m^{-2}$ on more than 60% of the sites, even the sites with the lowest crop biomass (Figure 5). At half of the sites, the biomass of sole-cropped lupin at flowering was less than 243 $g \cdot m^{-2}$. At this level of lupin biomass, weed growth was below 50 $g \cdot m^{-2}$ at a probability of less than 10% (Figure 5). At sites with a higher lupin biomass (higher than 243 $g \cdot m^{-2}$), weed biomass at lupin flowering could be maintained under 50 $g \cdot m^{-2}$ for 65% of those sites.

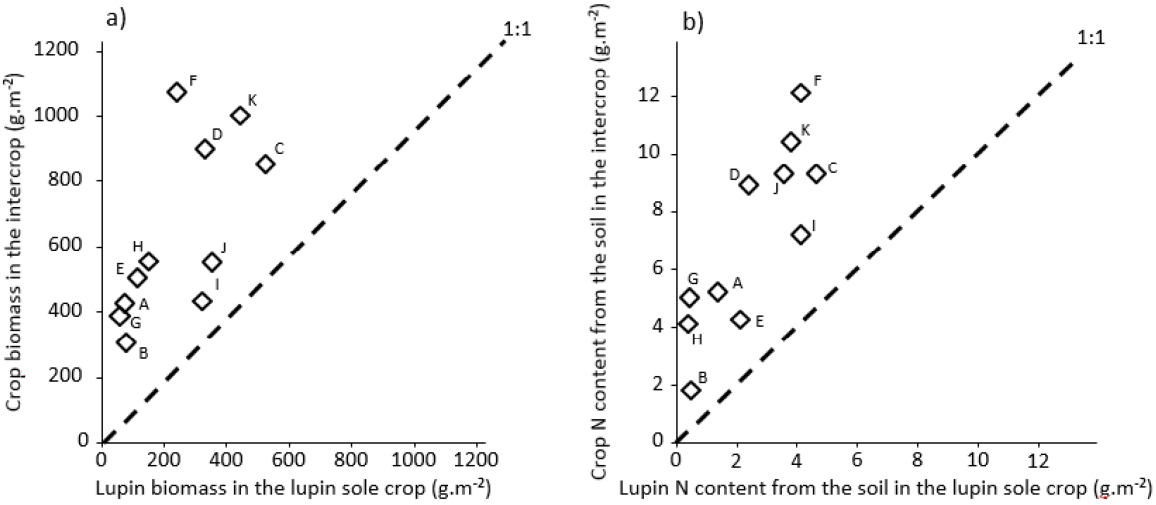

Figure 3. (**a**) Crop biomass in the intercrop against lupin biomass in the lupin sole crop at lupin flowering. (**b**) Crop N content from the soil in the intercrop against lupin N content from the soil in the lupin sole crop at lupin flowering. Letters next to points identify study sites. The dashed lines represent the theoretical situations with equal values in both cropping strategies.

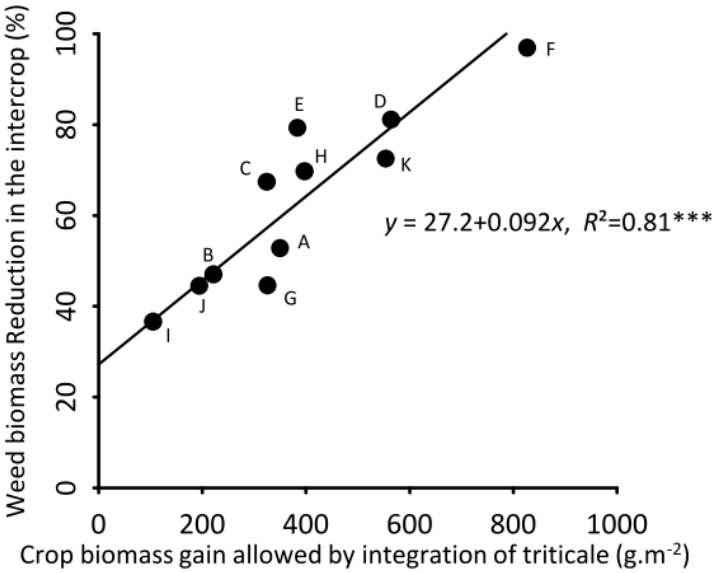

Figure 4. Correlation between weed biomass reduction in the intercrop compared to the lupin sole crop (WR) (%) and crop biomass gain allowed by addition of triticale at lupin flowering. Letters next to points identify study sites. *** indicate that the correlation is significant at $p < 0.001$.

From lupin flowering to maturity, the intercrop and the lupin sole crop produced similar amounts of DM. In the intercrop, the lupin biomass increased at a higher rate than did triticale biomass (+189% vs. +40%, respectively, Table 4). The variability of WR at maturity across sites was not explained by increases in crop or lupin biomass (no correlation, $p = 0.3$ and $p = 0.4$, respectively). However, the weed growth after lupin flowering was maintained at less than 60 g·m^{-2} when the crop biomass at lupin flowering attained the threshold value of 400 g·m^{-2} (Figure 6). This crop biomass value was attained in the intercrop at nine of eleven sites and in the lupin sole crop at two of eleven sites. The weed growth between lupin flowering and maturity was negative at some sites because of low weed growth and the decomposition of weed biomass in the end of the crop cycle.

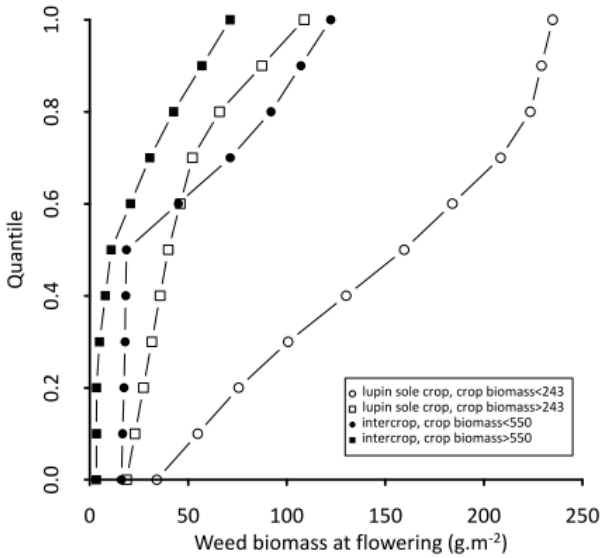

Figure 5. Distribution of weed biomass at lupin flowering across the 11 sites for different crop biomasses in the intercrop and in the lupin sole crop. Lupin sole crop and intercrop were separated in two pools according to median crop biomass so that for each cropping strategy, the sites with lowest crop biomass are represented with circles and the sites with highest crop biomass are represented with squares. Median crop biomass in the lupin sole crop: 243 g·m^{-2}, median crop biomass in the intercrop: 550 g·m^{-2}.

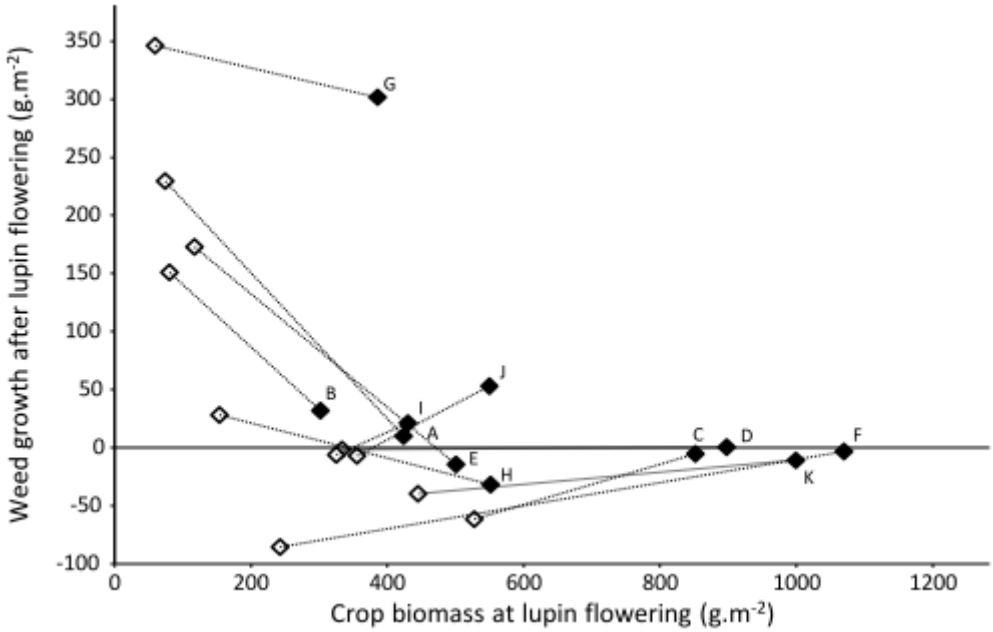

Figure 6. Weed growth after flowering against crop biomass at lupin flowering. Open symbols refer to lupin sole crops and full symbols refer to intercrops. Letters next to points identify study sites.

The mean proportion of triticale in the intercrop biomass was 75% at lupin flowering (CV: 14%) This proportion decreased to 59% at maturity and with a higher variability (CV = 28%). The addition of triticale reduced the proportion of weeds in total plant biomass. At flowering, the weed biomass represented 33% of the canopy in pure lupin and 7% in the intercrop. At maturity, the weed biomass represented 24% of the canopy in pure lupin and 8% in the intercrop.

The addition of triticale reduced weed biomass but also the diversity of weed species compared to lupin sole crop (Figure 7a). However, the proportion of mono/dicotyledonous species in the total number of weed species was not greatly modified except in site C (Figure 7b).

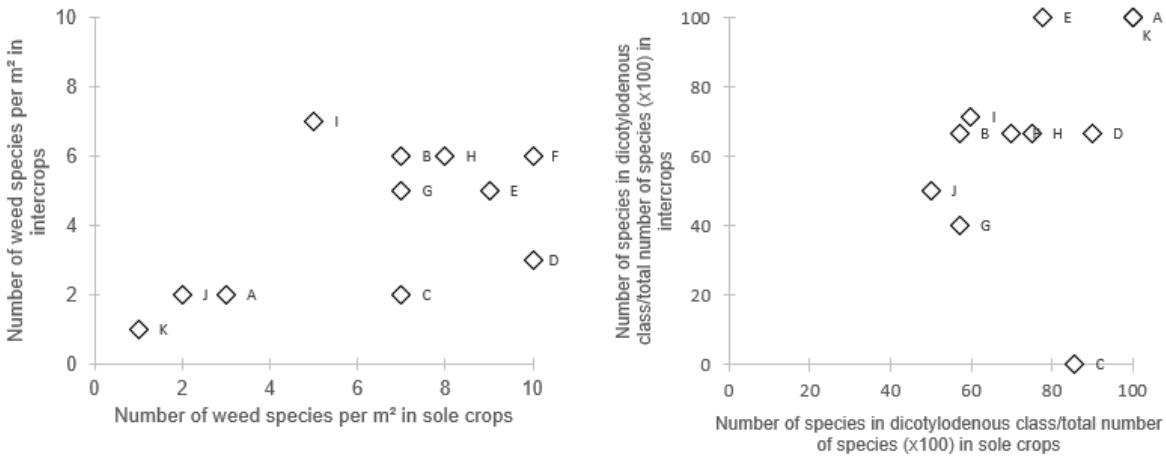

Figure 7. (**a**) Number of weed species in intercrops against number of weed species in lupin sole crops. (**b**) The percentage of dicotyledonous species in the total number of weed species in intercrops against the percentage in lupin sole crop. The dashed lines represent the theoretical situations with equal values in both cropping strategies.

3.2. Soil N Acquisition by Crops and Weeds before and after Flowering

Soil N acquisition was very low in lupin sole crop at the beginning of the crop cycle. It reached only 2.5 g·m^{-2} at the beginning of lupin flowering. Crop mineral soil N acquisition until lupin flowering was enhanced by the addition of triticale: the average crop mineral soil N acquisition gain was +4.5 g·m^{-2}, i.e., +181%, Table 4). In the intercrop, triticale acquired 88% of the crop mineral soil N acquired by the mixture until flowering. Triticale biomass was on average 3 times higher than lupin biomass and triticale soil mineral N acquisition was on average 8 times higher than lupin soil mineral N acquisition until flowering. Lupin acquired on average 5 mg of soil N per g of crop biomass produced, whereas triticale acquired 13 mg of soil N per g of crop biomass.

Weeds had a high ability to acquire soil mineral N: they acquired on average 19 mg of soil N per g of weed biomass at the beginning of lupin flowering. However, weed mineral soil N acquisition was reduced in the intercrop (−63% on average, Table 4) in comparison with weeds observed in the lupin sole crop. The lupin %Ndfa at the beginning of lupin flowering was significantly higher in the intercrop (84%, SD = 12) than in the lupin sole crop (66%, SD = 14, t_{10} = 3.0, p = 0.01; Table 5). The intercrop acquired more mineral soil N than did the lupin sole crop despite lupin depending less on mineral soil N in the intercrop.

At the end of winter, the integration of triticale had a tendency to reduce the mineral soil N content (53 kg·ha^{-1} in the sole crop and 43 kg·ha^{-1} in the intercrop, t_7 = −2.2, p = 0.06; Table 5), showing that an effect on the available N occurred rather early in the cropping season.

Total crop mineral soil N acquisition until lupin flowering varied less in the intercrop than in the lupin sole crop (Table 4). The CV for sole-cropped lupin was 64%, whereas the CV for the intercrop was 45%.

Table 5. Soil mineral N content and lupin %Ndfa (percentage of N accumulated in aboveground parts derived from N_2 fixation).

Site	Treatment	Soil Mineral N Content in the End of Winter (kg·ha^{-1})	Lupin %Ndfa at Flowering	Lupin %Ndfa at Maturity
A	Lupin SC	83	45	56
	IC	78	95	69
B	Lupin SC	18	77	79
	IC	n.d.	85	84
C	Lupin SC	72	75	69
	IC	45	81	74
D	Lupin SC	32	78	81
	IC	n.d.	95	100
E	Lupin SC	72	46	66
	IC	37	98	99
F	Lupin SC	86	49	62
	IC	38	80	75
G	Lupin SC	31	78	73
	IC	28	79	71
H	Lupin SC	25	89	71
	IC	25	94	76
I	Lupin SC	39	60	47
	IC	50	56	44
J	Lupin SC	61	68	24
	IC	43	74	37
K	Lupin SC	67	65	50
	IC	n.d.	85	70
mean	Lupin SC	53	66	62
	IC	43	84	73
SD	Lupin SC	25	15	17
	IC	16	12	19
comparison		$t_7 = -2.2$ n.s.	$t_{10} = 3.0$ *	$t_{10} = 3.4$ **

The significance levels of comparisons were assessed with *T*-tests. n.d.: not determined; n.s.: not significant. **, *, indicate significant differences among species at $p < 0.05$, $p < 0.01$, respectively.

In the lupin sole crop, only 27% of soil N was acquired before flowering, whereas in the intercrop, 67% of soil N was acquired before flowering. Thus, from lupin flowering until maturity, the lupin sole crop acquired more mineral soil N than did the intercrop (Table 4). As observed at lupin flowering, the lupin %Ndfa at maturity was significantly higher in the intercrop (73%, SD = 19) than in the lupin sole crop (62%, SD = 17, $t_{10} = 3.4$, $p = 6\ E^{-3}$; Table 5).

3.3. Grain and Productivity Yield of the Lupin Sole and Intercrop

In the lupin sole crop, the mean grain yield was 296 g·m^{-2} (minimum: 33 (site A), maximum: 663 (site K), Table 2), and the mean protein yield was 104 g·m^{-2}. The mean lupin yield was 34% lower in the intercrop than in the sole crop, but the effect of the addition of triticale on lupin yield differed among sites (Figure 8). Lupin yield was significantly lower in the intercrop than in the sole crop on four sites (Sites E, F, J and K) (Table 2). At sites A and B, two sites with low-to-medium lupin yields, the lupin grain yield tended to be higher in the intercrop than in the lupin sole crop. The lupin protein concentration did not significantly differ between the lupin sole crop and the intercrop (35.7% and 35.2%, respectively, with a CV of 17% in the sole crop and 15% in the intercrop; data not shown).

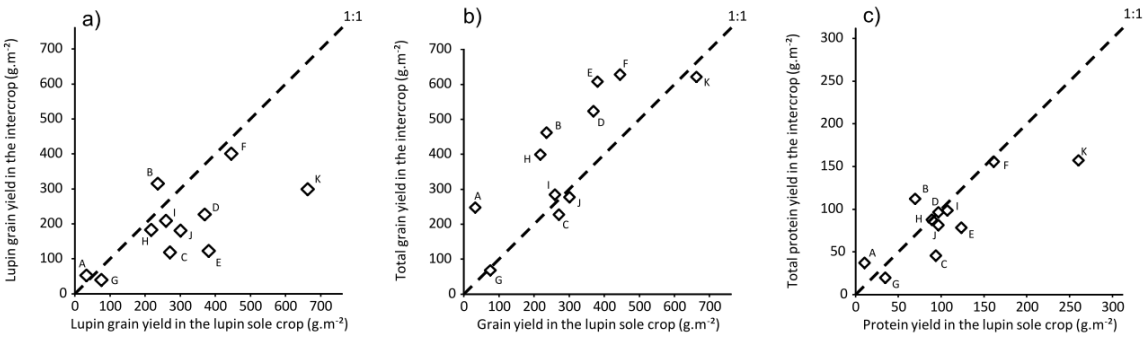

Figure 8. (**a**) Lupin grain yield in the intercrop against lupin grain yield in the lupin sole crop. (**b**) Total grain yield in the intercrop against lupin grain yield in the lupin sole crop. (**c**) Total protein yield in the intercrop against protein grain yield in the lupin sole crop. Letters next to points identify study sites. The dashed lines represent the theoretical situations with equal values in both cropping strategies.

Triticale produced on average 201 g·m^{-2} grain (minimum: 28 (site G), maximum: 485 (site E), Table 2), i.e., a similar yield as that of lupin in the intercrop (195 g·m^{-2} on average, t_{10} = 0.12, p = 0.9). Triticale grain had a mean protein concentration of 10.0% (data not shown). When considering total grain production, the intercrop produced more grain than did the lupin sole crop on average over all sites (+37%, mean: 395 g·m^{-2}, minimum: 68 (site G), maximum: 628 g·m^{-2} (site F)). At site A and site H, the lupin yield did not differ between the intercrop and the sole crop, but the total intercrop yield was significantly higher than the lupin sole crop yield (Table 2). At site A, the lupin grain yield was less than 100 g·m^{-2} in the sole crop and the addition of triticale allowed a total production of 248 g·m^{-2} grain (Table 2). At the four sites where the lupin yield was significantly lower in the intercrop than in the lupin sole crop, the intercrop total yield did not differ from the lupin sole crop yield (Table 2). The triticale proportion in intercrop grain biomass ranged from 27% to 80% (mean 49.5%).

On average, the total protein yield of the intercrop did not significantly differ from that of the lupin sole crop, but differences across sites were recorded (Figure 8). The reduction in protein production due to intercropping was highest at the site with the highest lupin yield in the sole crop (site K, −103 g·m^{-2} protein), as triticale protein production could not compensate for the reduction of protein-rich lupin grain production. However, on site B, the intercrop produced 43 g·m^{-2} more protein than did the lupin sole crop (Figure 8). In the intercrop, lupin represented on average 49% of grain yield and 78% of protein yield. The total grain and protein production values varied less across the eleven site-years in the intercrop than in the lupin sole crop (Table 2).

4. Discussion

Our results showed that the addition of triticale has a great ability to reduce weed biomass in lupin crops, especially when weed pressure is high, while maintaining protein yield. Weed biomass reduction can be explained by the increased crop biomass and mineral soil N acquisition especially at the beginning of the crop cycle through the addition of triticale. Moreover, crop biomass, mineral soil N acquisition, grain yield and protein yield were more stable in intercrops.

4.1. Weed Reduction Allowed by the Addition of Triticale and Underlying Processes

Weed reduction values at lupin flowering were consistently greater than 37% across a wide range of practices, pedo-climatic conditions and weed growth potentials (Figure 2, Table 2), demonstrating that the addition of triticale at 30% of its recommended density in the sole crop is effective at reducing weed growth. This finding is in line with previous studies on other intercropping systems. Focusing on intercrops that have a short growing season, Corre-Hellou et al. [16] studied spring pea–barley additive intercrops in which barley was sown at 150 kernels·m^{-2} (100:50) in five countries in Europe and obtained a mean WR of 55% at the beginning of pea flowering. Using an additive intercrop design

consisting of spring pea and oat (60 kernels·m^{-2}, 100:20), Gronle et al. [24] reported WR values of 14% and 27% at the beginning and end of pea flowering, respectively.

Weed growth in winter white lupin seems to utilize an available ecological niche related to the low growth rate of lupin until flowering. The addition of triticale in lupin occupied this niche; this occupation strongly and systematically increased crop weed suppression before flowering. Intercropping allowed attainment of crop biomass levels that ensure high weed control; this high crop biomass was rarely observed in lupin sole crops and is consistent with the finding that triticale can particularly compensate for the low competitive ability of a legume crop that produces low levels of crop biomass [24]. The less competitive a legume sole crop is, the more the addition of a cereal facilitates weed suppression.

In the lupin sole crop, mineral soil N not used by lupin was taken up by weeds, whereas in the intercrop, triticale acquired a large amount of mineral soil N to the detriment of weeds (Table 4). Cereals have a higher soil N requirement than legumes, and this demand is often associated with rapid root growth and a dense root system [9,25–28]. Integrating a cereal into a legume crop can result in the use of mineral soil N to produce cereal grain instead of weed biomass [15]. Lupin acquired much less soil mineral N in the intercrop than in the lupin sole crop due to the combined effects of higher %Ndfa and lower biomass, mitigating the effects of triticale soil mineral N acquisition. Hauggaard-Nielsen et al. [29] also reported that grain legumes accumulated less soil N when intercropped than was expected from sole crop acquisition.

Despite not being measured, competition for light is also assumed to be an important mechanism in the higher competitive ability of the intercrop than sole crop, as observed in other intercropping systems [30] due to complementary traits for leaf area distribution in the canopy and an increase in spatial homogeneity [31,32]. The spatial homogeneity may depend on sowing patterns of intercrops. Sowing the triticale in alternating rows in an additive design may decrease early heterogeneity of crop ground cover by filling the wide inter-row space in the lupin sole crop [33]. In our study, in the five sites (sites D, E, F, H, K) with highest WR (higher than 70%) at lupin flowering, intercrops were sown with triticale and lupin on alternating rows (Tables 1 and 2).

Lupin and triticale differ in their growth dynamics, and two contrasting periods were studied in the long crop cycle; the limit was the time of lupin flowering. Until lupin flowering, lupin had a low biomass production, which favored weeds in the lupin sole crop because the weeds could develop virtually without crop competition during autumn and winter and could gain an initial advantage over lupin during the first growing period (Tables 2 and 4). Triticale had a high biomass production during the initial growth stages (Tables 2 and 4). As reported in spring barley and pea [26,34], the maximum growth rate of the cereal occurred before that of the grain legume. Beginning at lupin flowering, the lupin growth rate strongly increased, whereas the triticale growth rate strongly decreased (Table 4). The clearly offset period of maximum growth rate and opposite growth patterns between lupin and triticale may allow temporal complementarity of resource use. In our study, compared with winter legume sole cropping, intercropping with triticale reduced weed growth before flowering but not after flowering (Table 4).

Complementarity of resource use in time and space in intercropping may be not the unique mechanism explaining weed suppression. Allelopathy effects can also contribute to weed suppression [35], but this mechanism was not investigated in our study and would require specific experiments. A better understanding on the mechanisms behind weed control and other benefits of intercropping systems can guide the design of various species combinations with traits that maximize positive or minimize negative interactions and reach expected services [36,37].

Weed species complexes encountered in the field network may have interacted with the effects of triticale. Nevertheless, the effect of intercropping seems effective for a wide range of weed compositions. Some weed species taller than triticale might limit the intercropping effect, but such situations were rare (only observed punctually during the crop cycle with *Erodium cicutarium* (site B), *Triplospermum inodorum* (site G), *Dactylis glomerata* (site G), and *Poa trivialis* (site J)). Our results indicated a reduction of the number of weed species in intercropping but without a modification of the repartition of the

species in monocotyledonous and dicotyledonous classes. Nevertheless, these results need to be confirmed with a wider range of situations and with additional data (plant density and biomass per weed species) to investigate more in depth the effect of intercropping on the structure of the weed community in relation to weed and crop functional traits.

4.2. Productivity of Intercropping Compared to Lupin Sole Cropping

This study confirmed the high potential of lupin to yield large amounts of grain and protein both in sole cropping and in intercropping systems. Although triticale reduced the lupin growth and yield, triticale grain production increased average total grain production by 37%. The mean lupin yield reduction by triticale (34%) was lower than that obtained by Hauggaard-Nielsen et al. [29] using a 50:50 substitutive design with narrow-leafed lupin and spring barley during a three-year period, during which intercropping reduced the lupin grain yield by 62%. It is likely that the lupin 100:triticale 30 additive design used in our study better maintains lupin yield than does a balanced substitutive design and still allows satisfactory cereal production. In our study, lupin compensated for the reduced soil N availability and fulfilled its N requirements by increasing the proportion of N derived from fixation; this mechanism is in agreement with the results of numerous studies [9,10,26,38,39]. However, the lupin biomass decreased, hence the reduced total N amount in lupin. The sites H and A showed promising results: despite triticale proportions in the intercrop biomass being higher than 80%, lupin yield was little decreased at site H (−16%) and even increased at site A (+60%). The reasons for these results are not clear, but they may have been in part favored by the sowing design, in which lupin and triticale were not sown on the same rows [40,41]. However, site A showed low lupin yields in both the sole crop and intercrop. A minimum of lupin plant density is important to reach satisfactory lupin yield and weed control levels: on the site with the lowest lupin density after winter (14 plants·m^{-2} in the lupin sole crop and in the intercrop, site G), lupin biomass at flowering and lupin grain yield in the intercrop were the lowest of all sites and weed biomass at maturity were by far the highest of all sites in the sole crop and in the intercrop.

Willey [42] reported that the growth of species sown in intercrops at the same density as that of sole crops is always less than the growth the sole crop. This phenomenon shows that full complementarity between intercropped species cannot be achieved. Lupin-triticale intercropping is a system in which the crop producing favored yield lets the companion crop have an initial advantage. This phenomenon is not ideal but seems inevitable when the main species exhibits slow early growth, as observed in grain legumes. In our study, this effect has possibly been further enhanced by the particularly mild autumn and winter during both study years. A lower temperature during crop establishment and early growth would probably have mitigated the cereal growth and dominance in the intercrop because it would have delayed the beginning of cereal maximal growth phase and reduced tillering, whereas lupin maximal growth phase and branching takes place later and would not have been affected.

Grain yield variability across a wide range of situations was lower when considering total intercrop yield rather than lupin sole crop yield. This result is consistent with previous results on both spring intercrops [9] and winter intercrops [43] but contrasts with those of Hauggaard-Nielsen et al. [29], who reported no yield stability differences between narrow-leafed (spring) lupin-barley intercrops and narrow-leafed lupin sole crops. The higher yield stability measured in this study needs to be assessed in long-term studies. The level of yield variability remained high in the intercrop; however, here, we mostly characterized between-site variability, whereas farmers may be more interested in ways to increase inter-annual stability. If for lupin, intercropping proves to be an efficient way to secure yield, this could be a convincing argument for some farmers who could decide to replace lupin sole crop with lupin–cereal intercrop or start to grow lupin using the intercropping strategy.

4.3. Perspectives for the Use of Lupin-Based Intercrops

Effective weed control combined with the maintenance of lupin yield in the intercrop occurred for instance on site F where the combination of increased total crop biomass and a high proportion

of lupin in the crop biomass occurred. In situations where sole-cropped lupin can perform well (limited biotic and abiotic stresses under a favorable climate, the absence of water logging and the use of pesticides and herbicides, e.g., site K in our study), adding a cereal will very likely reduce protein yields. However, intercropping has a high potential for lupin growth in suboptimal conditions that are becoming increasingly frequent and unpredictable due to climate change. Intercropping should also be promoted as part of an integrated agronomic strategy in combination with other agronomic measures such as crop rotation, cover cropping and mechanical weeding to limit or forfeit the use of herbicides. Adding cereals in grain legume crops seems to allow maintaining protein productivity while keeping weed biomass within acceptable thresholds without or with a low use of herbicides. This additive intercropping design should therefore be promoted as a strategy to facilitate production of grain legumes following the need to reduce the use of herbicides, in the same way as other (mostly substitutive) intercropping designs have been promoted as strategies to increase total grain production and cereal protein concentration in low-input systems [44,45].

Although the triticale cultivars and density levels used in this study showed good performances, further adjustment of cereal species or cultivar choice or density fine-tuning is needed, as competition from the companion crop on lupin must be reduced. We hypothesize that on two sites (F and H), the alternating row design played a role in allowing the competition of triticale against lupin to be lower than that at other sites that had the same level of triticale proportion in the intercrop biomass. Specific experiments are needed to compare different spatial arrangements. Triticale cultivars or other cereal species with a shorter height after stem elongation may be favorable to maintain lupin yield. Selecting lupin cultivars for traits best adapted to intercropping with cereals could further increase the benefits of this cropping strategy [46].

Most farmers and experimenters managing experimental fields had no previous experience in lupin intercropping, suggesting that large room for optimization of field choice and management practices exists and that higher performances of the intercrop can be expected. In our field network, sole-cropped lupin management was not optimal since practices adapted to the intercrop were applied to both cropping strategies. Specifically, in lupin sole crops, a post-emergence herbicide treatment was typically applied in conventional fields at the time of the study and it has not been used here in eight of the eleven sites. The potential of sole-cropped lupin may have been underestimated in this study.

5. Conclusions

Comparing the intercrop and the sole crop in the context of the transition to low-input crop management strategies is increasingly needed as solutions for chemical weeding are becoming scarce. In this context, we showed that the lupin-triticale intercrop is a relevant option. Because a moderate lupin yield reduction can lead to a high protein yield loss, intercropping lupin with triticale does not seem to potentially perform better than sole cropping lupin regarding protein productivity on an area basis. At a broader scale, intercropping could allow an increase in lupin cropping area via increased lupin adoption by farmers due to increased weed suppression and secured total productivity. In this case, lupin intercropped with cereals could significantly contribute to the production of protein-rich grains in Europe.

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References

1. De Visser, C.L.; Schreuder, R.; Stoddard, F. The EU's dependency on soya bean import for the animal feed industry and potential for EU produced alternatives. *OCL* **2014**, *21*, D407. [CrossRef]
2. Van Barneveld, R.J. Understanding the nutritional chemistry of lupin (*Lupinus* spp.) seed to improve livestock production efficiency. *Nutr. Res. Rev.* **1999**, *12*, 203–230. [CrossRef] [PubMed]
3. Lucas, M.M.; Stoddard, F.; Annicchiarico, P.; Frias, J.; Martinez-Villaluenga, C.; Sussmann, D.; Duranti, M.; Seger, A.; Zander, P.; Pueyo, J. The future of lupin as a protein crop in Europe. *Front. Plant Sci.* **2015**, *6*, 705. [CrossRef] [PubMed]
4. Herridge, D.F.; Peoples, M.B.; Boddey, R.M. Global inputs of biological nitrogen fixation in agricultural systems. *Plant Soil* **2008**, *311*, 1–18. [CrossRef]
5. FAO. FAOSTAT. (Ed Food and Agriculture Organization of the United Nations). 2017. Available online: www.Fao.org/faostat/en/# (accessed on 3 August 2017).
6. Cernay, C.; Ben-Ari, T.; Pelzer, E.; Meynard, J.M.; Makowski, D. Estimating variability in grain legume yields across Europe and the Americas. *Sci. Rep.* **2015**, *5*, 11171. [CrossRef]
7. Putnam, D.H.; Wright, J.; Field, L.A.; Ayisi, K.K. Seed yield and water-use efficiency of white lupin as influenced by irrigation, row spacing, and weeds. *Agron. J.* **1992**, *84*, 557–563. [CrossRef]
8. Folgart, A.; Price, A.; Van Santen, E.; Wehtje, G. Organic weed control in white lupin (*Lupinus albus*, L.). *Renew. Agr. Food Syst.* **2011**, *26*, 193–199. [CrossRef]
9. Jensen, E.S. Grain yield, symbiotic N_2 fixation and interspecific competition for inorganic N in pea-barley intercrops. *Plant Soil* **1996**, *182*, 25–38. [CrossRef]
10. Hauggaard-Nielsen, H.; Gooding, M.; Ambus, P.; Corre-Hellou, G.; Crozat, Y.; Dahlmann, C.; Dibet, A.; von Fragstein, P.; Pristeri, A.; Monti, M.; et al. Pea–barley intercropping for efficient symbiotic N_2-fixation, soil N acquisition and use of other nutrients in European organic cropping systems. *Field Crop. Res.* **2009**, *113*, 64–71. [CrossRef]
11. Bedoussac, L.; Journet, E.P.; Hauggaard-Nielsen, H.; Naudin, C.; Corre-Hellou, G.; Jensen, E.S.; Prieur, L.; Justes, E. Ecological principles underlying the increase of productivity achieved by cereal-grain legume intercrops in organic farming. A review. *Agron. Sustain. Dev.* **2015**, *35*, 911–935. [CrossRef]
12. Risch, S.J. Intercropping as cultural pest control: Prospects and limitations. *Environ. Manag.* **1983**, *7*, 9–14. [CrossRef]
13. Boudreau, M.A. Diseases in Intercropping Systems. *Annu. Rev. Phytopathol.* **2013**, *51*, 499–519. [CrossRef] [PubMed]
14. Liebman, M.; Dyck, E. Crop rotation and intercropping strategies for weed management. *Ecol. Appl.* **1993**, *3*, 92–122. [CrossRef] [PubMed]
15. Hauggaard-Nielsen, H.; Ambus, P.; Jensen, E.S. Interspecific competition, N use and interference with weeds in pea-barley intercropping. *Field Crop. Res.* **2001**, *70*, 101–109. [CrossRef]
16. Corre-Hellou, G.; Dibet, A.; Hauggaard-Nielsen, H.; Crozat, Y.; Gooding, M.; Ambus, P.; Dahlmann, C.; von Fragstein, P.; Pristeri, A.; Monti, M.; et al. The competitive ability of pea-barley intercrops against weeds and the interactions with crop productivity and soil N availability. *Field Crop. Res.* **2011**, *122*, 264–272. [CrossRef]
17. Bohm, H.; Aulrich, K. Effects of row distances and seed densities on yield and quality of blue lupin (L. Augustifolius) in organic farming. In *Proceedings of the 13th International Lupin Conference, Poznan, Poland*; Naganowska, B., Kachlicki, P., Wolko, B., Eds.; International Lupin Association: Temuco, Chile, 2011.
18. Carton, N.; Naudin, C.; Piva, G.; Corre-Hellou, G. Variability of traits associated with early N acquisition in lupin and early complementarity in lupin-triticale mixed stands. *Aob. Plants* **2018**, *10*, ply001. [CrossRef]

19. ISO. *Soil quality—Determination of Nitrate, Nitrite and Ammonium in Field-Moist Soils by Extraction with Potassium Chloride Solution—Part 2: Automated Method with Segmented Flow Analysis*; International Organization for Standardization: Geneva, Switzerland, 2005.
20. Amarger, N.; Mariotti, A.; Mariotti, F.; Durr, J.C.; Bourguignon, C.; Lagacherie, B. Estimate of symbiotically fixed nitrogen in field grown soybeans using variations in 15N Natural abundance. *Plant Soil* **1979**, *52*, 269–280. [CrossRef]
21. Komsta, L. Outliers: Tests for Outliers. Available online: http://www.komsta.net/ (accessed on 24 January 2011).
22. Benjamini, Y.; Hochberg, Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B (Methodol.)* **1995**, *57*, 289–300. [CrossRef]
23. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2013; Available online: http://www.R-project.org/ (accessed on 2 September 2016).
24. Gronle, A.; Böhm, H.; Heß, J. Effect of intercropping winter peas of differing leaf type and time of flowering on annual weed infestation in deep and shallow ploughed soils and on pea pests. *Landbauforschung Volkenrode.* **2014**, *64*, 31–44. [CrossRef]
25. Hauggaard-Nielsen, H.; Ambus, P.; Jensen, E.S. Temporal and spatial distribution of roots and competition for nitrogen in pea-barley intercrops—A field study employing 32P technique. *Plant Soil* **2001**, *236*, 63–74. [CrossRef]
26. Andersen, M.K.; Hauggaard-Nielsen, H.; Ambus, P.; Jensen, E.S. Biomass production, symbiotic nitrogen fixation and inorganic N use in dual and tri-component annual intercrops. *Plant Soil* **2004**, *266*, 273–287. [CrossRef]
27. Corre-Hellou, G.; Crozat, Y. Assessment of Root System Dynamics of Species Grown in Mixtures under Field Conditions using Herbicide Injection and 15N Natural Abundance Methods: A Case Study with Pea, Barley and Mustard. *Plant Soil* **2005**, *276*, 177–192. [CrossRef]
28. Corre-Hellou, G.; Brisson, N.; Launay, M.; Fustec, J.; Crozat, Y. Effect of root depth penetration on soil nitrogen competitive interactions and dry matter production in pea–barley intercrops given different soil nitrogen supplies. *Field Crop. Res.* **2007**, *103*, 76–85. [CrossRef]
29. Hauggaard-Nielsen, H.; Jørnsgaard, B.; Kinane, J.; Jensen, E.S. Grain legume–cereal intercropping: The practical application of diversity, competition and facilitation in arable and organic cropping systems. *Renew. Agric. Food Syst.* **2008**, *23*, 3–12. [CrossRef]
30. Abraham, C.; Singh, S. Weed management in sorghum-legume intercropping systems. *J. Agric. Sci.* **1984**, *103*, 103–115. [CrossRef]
31. Schwinning, S.; Weiner, J. Mechanisms determining the degree of size asymmetry in competition among plants. *Oecologia* **1998**, *113*, 447–455. [CrossRef]
32. Weiner, J.; Griepentrog, H.W.; Kristensen, L. Suppression of weeds by spring wheat *Triticum aestivum* increases with crop density and spatial uniformity. *J. Appl. Ecol.* **2001**, *38*, 784–790. [CrossRef]
33. Olsen, J.M.; Griepentrog, H.W.; Nielsen, J.; Weiner, J. How important are crop spatial pattern and density for weed suppression by spring wheat? *Weed Sci.* **2012**, *60*, 501–509. [CrossRef]
34. Bellostas, N.; Hauggaard-Nielsen, H.; Andersen, M.K.; Jensen, E.S. Early interference dynamics in intercrops of pea, barley and oilseed rape. *Biol. Agric. Hortic.* **2003**, *21*, 337–348. [CrossRef]
35. Reiss, A.; Fomsgaard, I.S.; Mathiassen, S.K.; Kudsk, P. Weed suppressive traits of winter cereals: Allelopathy and competition. *Biochem. Syst. Ecol.* **2018**, *76*, 35–41. [CrossRef]
36. Brooker, R.W.; Bennett, A.E.; Cong, W.F.; Daniell, T.J.; George, T.S.; Hallett, P.D.; Hawes, C.; Iannetta, P.P.M.; Jones, H.G.; Karley, A.J.; et al. Improving intercropping: A synthesis of research in agronomy, plant physiology and ecology. *New Phytol.* **2015**, *206*, 107–117. [CrossRef] [PubMed]
37. Damour, G.; Dorel, M.; Quoc, H.T.; Meynard, C.; Risède, J.M. A trait-based characterization of cover plants to assess their potential to provide a set of ecological services in banana cropping systems. *Eur. J. Agron.* **2014**, *52*, 218–228. [CrossRef]
38. Corre-Hellou, G.; Fustec, J.; Crozat, Y. Interspecific competition for soil N and its interaction with N_2 fixation, leaf expansion and crop growth in pea-barley intercrops. *Plant Soil* **2006**, *282*, 195–208. [CrossRef]
39. Naudin, C.; Corre-Hellou, G.; Pineau, S.; Crozat, Y.; Jeuffroy, M.H. The effect of various dynamics of N availability on winter pea-wheat intercrops: Crop growth, N partitioning and symbiotic N_2 fixation. *Field Crop. Res.* **2010**, *119*, 2–11. [CrossRef]

40. Yunusa, I. Effects of planting density and plant arrangement pattern on growth and yields of maize (*Zea mays*, L.) and soya bean (*Glycine max* (L.) Merr.) grown in mixtures. *J. Agric. Sci.* **1989**, *112*, 1–8. [CrossRef]
41. Kristensen, L.; Olsen, J.; Weiner, J. Crop density, sowing pattern, and nitrogen fertilization effects on weed suppression and yield in spring wheat. *Weed Sci.* **2008**, *56*, 97–102. [CrossRef]
42. Willey, R. Intercropping—its importance and research needs: Part 1. Competition and yield advantages. *Field Crop Abstr.* **1979**, *32*, 1–10.
43. Naudin, C.; Aveline, A.; Corre-Hellou, G.; Dibet, A.; Jeuffroy, M.H.; Crozat, Y. Agronomic analysis of the performance of spring and winter cereal-legume intercrops in organic farming. *J. Agric. Sci. Technol.* **2009**, *3*, 17–28.
44. Gooding, M.J.; Kasyanova, E.; Ruske, R.; Hauggaard-Nielsen, H.; Jensen, E.S.; Dahlmann, C.; von Fragstein, P.; Dibet, A.; Corre-Hellou, G.; Crozat, Y.; et al. Intercropping with pulses to concentrate nitrogen and sulphur in wheat. *J. Agric. Sci.* **2007**, *145*, 469–479. [CrossRef]
45. Bedoussac, L.; Justes, E. The efficiency of a durum wheat-winter pea intercrop to improve yield and wheat grain protein concentration depends on N availability during early growth. *Plant Soil* **2010**, *330*, 19–35. [CrossRef]
46. Hauggaard-Nielsen, H.; Jensen, E.S. Evaluating pea and barley cultivars for complementarity in intercropping at different levels of soil N availability. *Field Crop. Res.* **2001**, *72*, 185–186. [CrossRef]

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