Field Crops Research 145 (2013) 78–87

Contents lists available at SciVerse ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Relay intercropping of legume cover crops in organic winter wheat: Effects on performance and resource availability

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ARTICLE INFO

Article history:
Received 24 April 2012
Received in revised form
14 November 2012
Accepted 16 February 2013

Keywords:
Intercropping
Grain protein content
Grain yield
Soil water content
Nitrogen uptake
Intercepted radiation
Organic farming

ABSTRACT

Wheat yields and grain protein content are lower and more variable in organic conditions than in conventional agriculture, mainly due to nitrogen (N) deficiency and weed competition. The undersowing of legume cover crops in growing winter wheat, also known as relay intercropping, is assumed to be a proficient way of enriching the soil-crop system with N and improving weed control. However, competition for resources may impair wheat performance. Relay intercropping in springtime in growing winter wheat is expected to limit competition for resources from the legume. The aim of our study was to analyze wheat performance and resource competition in relay intercropping systems with legume cover crops under various environmental conditions.

Eight field experiments were conducted on organic grain-oriented farms located in southeast France. Black medic (*Medicago lupulina*), alfalfa (*Medicago sativa*), red clover (*Trifolium pratense*) and white clover (*Trifolium repens*), undersown at wheat tillering stage, were compared with a sole crop of winter wheat (*Triticum aestivum* cv. Lona). The aerial biomass of wheat and legumes and total N content of wheat were determined at both the flowering stage and harvest time of the wheat. The main trophic resources (water, N and light) were monitored at similar stages. Wheat grain yield and grain protein content were also measured. Sites were classified according to wheat sole crop performance at harvest to evaluate the effect of relay intercropping in the various conditions tested.

In all but one of the thirty-two treatments, wheat grain yield was not significantly disturbed by relay intercropping whatever the aerial development of legumes. Abundant aerial development of legumes after flowering (>20% of the aerial biomass of wheat at harvest) was associated with a mean significant decrease in wheat grain protein content of 8.5% of the control treatment value, this occurring in one third of the situations. These ratios of aerial biomasses of legumes compared to wheat were also associated with reductions in soil moisture and wheat N uptake. The effect on the availability of trophic resources for wheat may have led to a decrease in grain protein content without having any effect on grain yield. Sites which registered a low performance for wheat sole crops were less negatively impacted by the presence and aerial development of legumes.

The performance of the four legume species and the mechanisms of induced disturbance of resource availability are then discussed. Finally, we try to define ranges of legumes' development and resource availability in which wheat performance was maintained or limited.

1. Introduction

Many studies on organic grain production identify legume crops as a proficient way of providing nitrogen (N) to high N-demanding grain crops (Goewie, 2002; Robson et al., 2002; Mazzoncini et al., 2004; Casagrande et al., 2009). Their capacity to fix atmospheric N and make it available to non-fixing plants (Askegaard and Eriksen, 2007; Fustec et al., 2010) has made them of increasing interest in organic grain systems subject to N deficiency (David et al., 2005a).

Furthermore weeds are often recognized as the most serious threat to organic crop production (Bàrberi, 2002). The use of legume crops as cover crops between crop plants (Hiltbrunner et al., 2007) or grown in the period between two cash crops (Kruidhof et al., 2008) has been shown to be efficient on weed control. As living plants or plant residues, they decrease light intercepted by weeds and limit their emergence (Teasdale et al., 2007). The use of legume crops can also provide other long-term agro-ecological services

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http://dx.doi.org/10.1016/j.fcr.2013.02.010

such as biodiversity enhancement or soil protection (Hartwig and Ammon, 2002; Sheaffer and Seguin, 2003).

N availability for the subsequent and/or associated cash crops largely depends on the N accumulation provided by the legume crops, either as cover crops or cash crops, mostly linked to their production of biomass (Olesen et al., 2009; Bergkvist et al., 2011). Forage legumes cropped as green manure potentially provide higher N amounts to the cropping system than legumes cropped for grains (Ma et al., 2003; Sheaffer and Seguin, 2003), since harvested seeds can concentrate more than 75% of N accumulated in the plant (Mahieu et al., 2007).

In organic stockless systems, medium-term legume leys are shown to be less economically viable than grain crops (Robson et al., 2002; David, 2009). Intercropping legume cover crops with a cash crop could be an interesting option for improving N fertility while maintaining a cash crop every year (Thiessen Martens et al., 2001). Moreover, cereal-legume cover crop intercrops may improve radiation use, thanks to different aerial architectures and the life cycle timing of both crops (Shili-Touzi et al., 2010). The intercropping system can also improve N availability through complementarity in nutrient sources from soil and N fixation (Tosti et al., 2010). Finally, the modification of the root architecture due to competition for soil resources could improve water and nutrient uptake by the cash crop (Li et al., 2011).

Resource competition by the legume cover is expected to limit weed development (Teasdale et al., 2007). In intercropping situations with legume living mulches, competition for resources had also negative consequences on cash crops (Carof et al., 2007a; Hiltbrunner et al., 2007). Competition, here, may take the form of light interception due to shading of the cash crop by the cover crop especially in the early stages of cash crop growth as shown by Carof et al. (2007a,b) for wheat sown in legume living mulches. Using a similar wheat species and living white clover intercrop, Thorsted et al. (2006) observed competition on soil water availability, while nutrient resources were the main source of competition between wheat and legume cover crops.

In intercropping systems combining cereals and legume cover crops, the dominance of cereals over legumes is essential to avoid resource limitation to the cash crop, thereby inducing limited performance (Andersen et al., 2007; Carof et al., 2007b; Shili-Touzi et al., 2010). Establishing cereal growth prior to the development of the legume cover crop, by delaying the legume sowing date or by using species with different growing cycles, can ensure that the cereal enjoys better access to trophic resources. Relay intercropping of legume cover crops (RIL), by delaying the sowing of the legume until springtime, is expected (i) to ensure that the winter cash crop is sufficiently established and dominant to limit the opportunity of competition with the cover crop thereby inducing limited cereal performance but (ii) without inhibiting their emergence and growth, as proposed by Shili-Touzi et al. (2010) after modeling of winter wheat-red fescue intercropping in conventional agriculture over 35 climatic years.

Several studies undertaken in conventional agriculture on RIL in wheat, aimed at optimizing cover crop development by testing crop density (Mutch et al., 2003; Blaser et al., 2006, 2007; Queen et al., 2009), legume cultivars (Mutch et al., 2003; Singer et al., 2006), fertilization rates (Queen et al., 2009) or sowing techniques, by comparing broadcast and drill seeding for instance (Edwards, 1998). The previous research that was interested in the final performance of the relay intercropped cereal crop has demonstrated that RIL in winter wheat has no negative effect on conventional (Thiessen Martens et al., 2001; Mutch et al., 2003; Blackshaw et al., 2010; Bergkvist et al., 2011) or organic wheat grain yield (Hartl, 1989; Becker and Leithold, 2008; Olesen et al., 2009). However, few studies have evaluated the impact of RIL on wheat grain protein content. Becker and Leithold (2008) concluded that a mixture of legumes cover crops undersown in early spring in 50 cm wheat inter-rows had no effect and Blackshaw et al. (2010) observed the same result with red clover or alfalfa spring-planted in winter wheat. The literature is even less abundant about RIL when we consider organic conditions (Hartl, 1989; Becker and Leithold, 2008; Olesen et al., 2009) in which nutrient resources are frequently limited.

Most studies on RIL have focused on hardy winter legume species like red or white clovers tested in conventional agriculture and developed under cold climate conditions (northern or eastern Europe, northern United-States and southern Canada) (Peel et al., 2007). In temperate conditions, other legume cover crop species could be suitable.

While it is assumed that the performance of the cash crop can be linked to resource competition between the two crops, little attention has been paid to this relation in RIL systems. Thus, resource monitoring is expected to help understand possible perturbations of the performance of relay intercropped winter wheat.

The general aims of our study were (i) to evaluate the establishment of various relay intercropped legumes cover crops in organic winter wheat, (ii) to evaluate their incidence on wheat grain yield and grain protein content, and (iii) to analyze the effect of relay intercropped legumes on resource availability (water, N and light), in diverse conditions of cash crop development, soil and weather.

2. Material and methods

2.1. Experimental sites

Eight field experiments (named hereafter as sites) were carried out, four in crop season 2008–2009 (A09, B09, C09 and D09) and four in 2009–2010 (E10, F10, G10 and H10) (Table 1). The field experiments were set up on organic farms located in south-east France over a large area, around 6000 km^2 (44°6′–45°7′N and 4°8′–5°6′E) representing various climatic conditions from continental to Mediterranean influences. Mean temperatures during the wheat crop cycle (from sowing to harvest time) ranged from 8.7 to 10.9 °C (Table 1).

Rainfall regimes were variable over years and sites. Cumulative precipitation during the wheat crop cycle ranged between 401 and 784 mm (Table 1). Additional irrigation was applied once or twice from the beginning of April (wheat growth stage, GS 23) (Zadoks et al., 1974) to the end of May (GS 69). The water balance of the crop cycle, calculated as the difference between precipitation plus irrigation (in mm) and potential evapotranspiration (in mm) ranged from −183 to +291 mm.

The field experiments were characterized by various soil types (FAO, 2006) (Table 1): three silt loam soils (D09, F10 and H10), two sandy loam (C09 and E10) and three with a higher rate of clay over 25% (A09, B09 and G10). Soil total N largely ranged from 0.9 to 2.5 g kg^{-1}. Maximum available soil water content of the 0–90 cm layer, calculated using the soil-plant-air-water (SPAW) model developed by Saxton et al. (2006) and based on simple textural soil characteristics, ranged from 130 to 240 mm.

Sites were also characterized by various weed infestations during the crop cycle. For instance, the ratio of weed/wheat aerial dry matter, expressed at wheat harvest time on wheat sole crop treatment, ranged from 0.2% to 6.6% (Table 1). High weed infestations exceeding 5% occurred on stony calcareous soils. All mechanical weed control was carried out with a tined weeder before cover crop sowing. All weed controls were performed before the legumes were sown, even on the wheat sole crop treatment. The last weed control

Table 1
Soil and climatic conditions and crop management of the field experiments.

Site codes	A09	B09	C09	D09	E10	F10	G10	H10
Location	44°37′N, 5°26′E	44°46′N, 5°21′E	44°45′N, 4°56′E	45°38′N, 4°54′E	44°45′N, 4°57′E	45°39′N, 4°53′E	45°41′N, 5°32′E	45°13′N, 4°49′E
Climate from sowing of wheat to harvest								
Cumulative rainfall and irrigation (mm)	544	611	544 (70)[d]	401	646 (70)[d]	591 (40)[d]	784	506
Mean temperature (°C)	8.7	9.3	10.9	10.2	10.7	10.3	9.7	10.2
Water balance (mm)[a]	−47	53	−93	−183	−2	−51	291	−113
Soil								
Texture[b]	CL (C 27% S 21%)	SiCL (C 37% S 10%)	SL (C 15% S 63%)	SiL (C 17% S 25%)	SL (C 16% S 60%)	SiL (C 15% S 14%)	SiC (C 43% S 8%)	SiL (C 20% S 27%)
Available soil water content on 90 cm depth (mm)[c]	200	180	130	230	140	240	160	215
Total nitrogen ($g\,kg^{-1}$)	2.0	2.1	1.0	0.9	1.1	0.9	2.5	1.0
Weed infestation at harvest on control treatment								
Weed/Wheat aerial DM ratio (in %)	6.1	6.6	0.8	0.2	1.9	3.6	2.0	0.3
Preceding crop and principal dates								
Preceding crop	Winter wheat	Winter wheat	Soybean	Vetch	Soybean	Corn	Soybean	Soybean
Wheat sowing	2/11/08	26/10/08	26/11/08	20/11/08	13/11/09	27/10/09	30/10/09	26/10/09
Wheat harvesting	9/7/09	10/7/09	9/7/09	6/7/09	13/7/10	12/7/10	16/7/10	13/7/10
Legumes sowing	25/3/09	25/3/09	26/3/09	26/3/09	18/3/09	15/3/10	15/3/10	17/3/10

[a] Water balance = (rainfall + irrigation) – potential evapotranspiration.
[b] Soil classification from FAO (2006): CL = clay loam; SL = sandy loam; SiC = silty clay; SiCL = silty clay loam; SiL = silty loam; C = clay; S = sand.
[c] Method defined by Saxton et al. (2006).
[d] Including irrigation in brackets (in mm).

on all the fields was also used to bury legume seeds the same day or one to two days after their sowing.

In all fields, winter wheat (cv. Lona) was sown at a rate of 200 kg ha^{-1}. The dates of sowing and the nature of the preceding crops are given in Table 1.

2.2. Experimental design and treatments

Four species of undersown legumes were compared. Black medic (*Medicago lupulina* L.), alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.) and white clover (*Trifolium repens* L.) were broadcast sown (800 seeds m^{-2}) with a manual centrifugal seed spreader at the end of wheat tillering (GS 22) (Table 1). An additional control treatment with wheat grown as sole crop referred to as T0 was implemented to evaluate the effect of undersown legumes. Legume covers were maintained after wheat grain harvest and then ploughed-in at the end of winter. Each treatment was repeated in three randomized blocks per site. The size of each treatment was approximately 200 m^2.

No additional fertilization was performed at springtime so as not to interfere with the sole effect of legumes on wheat N nutrition and crop performance.

2.3. Data collection

2.3.1. Plant development and wheat performance

Wheat, legume and weed aerial dry matter (DM, kg ha^{-1}) were measured at the wheat flowering stage (GS 69) and just before wheat harvest by the farmers, close to wheat maturity stage (GS 92). Three samples of 0.25 m^2 per treatment were hand harvested at each sampling date. The three components, wheat, legumes and weeds, were separated and weighed after oven-drying (48 h, 60 °C in air oven). At harvest, ears and straw were weighed separately. Then the ears were threshed and the grains were weighed after oven-drying (48 h, 60 °C in air oven). Grain yield data was adjusted to 15% moisture content. Total grain protein content (GPC in % DM) was determined using size-exclusion high-performance liquid chromatography (Morel et al., 2000).

2.3.2. Soil moisture

Three soil samples corresponding to the soil layers 0–30 cm, 30–60 cm and 60–90 cm were taken on each treatment at wheat harvest. Each sample was obtained by mixing three sub-samples per treatment. The water content of each sample (in %) was calculated as the ratio between water mass in soil samples and dry soil sample mass after oven-drying (48 h, 105 °C in air oven). Soil moisture on the whole soil profile was calculated as the mean mass humidity of the three layers per treatment.

2.3.3. Wheat nitrogen content

The three wheat samples of 0.25 m^2 per treatment were gathered at the wheat flowering stage (GS 69) and ground using a 1 mm mesh grid to determine the wheat N content of each treatment. A sub-sample of 5.0–7.5 mg of homogenous material was analyzed for total N concentration using mass spectrometry (Delta Plus Avantage, Thermo-Electron, Bremen, Germany). Wheat N uptake (kg N ha^{-1}) was calculated at wheat flowering multiplying aerial dry biomass by total N concentration. Wheat N accumulation at wheat harvest was calculated using the measured N content in grains and assuming a constant N harvest index (Austin and Jones, 1975) fixed at 0.76 (Barbottin et al., 2005). N uptake in the aerial parts was multiplied by 1.25 to estimate the total N quantity accumulated by wheat, including roots, at both dates (Coïc, 1956).

2.3.4. Radiation interception

Photosynthetically active radiation (PAR in μmol m^{-2} s^{-1}) intercepted by the whole canopy was determined at wheat flowering (GS 69) and harvest using an AccuPAR LP-80 ceptometer (Decagon Devices, Pullman, WA, USA). Measurements were obtained when incident radiation greater than 600 μmol m^{-2} s^{-1} was observed above the wheat, measured 3 h before and after the zenith.

The data were obtained by placing the ceptometer across 6 wheat rows with 6 measurements at ground level in each treatment. An additional sensor was placed above the wheat canopy to measure total incident radiation simultaneously with each ground measurement. The proportion of incident radiation intercepted by the total vegetation from above the wheat canopy to ground level, including legumes, weeds and wheat was calculated using the following equation:

$$\mathrm{PAR}i = \frac{\mathrm{PAR}_A - \mathrm{PAR}_B}{\mathrm{PAR}_A} \times 100$$

where PAR*i* (%) is the proportion of total incident PAR intercepted, $\mathrm{PAR_A}$ (in μmol m^{-2} s^{-1}) the total incident radiation above wheat and $\mathrm{PAR_B}$ (in μmol m^{-2} s^{-1}), the radiation at ground level.

2.4. Site classification

To take advantage of site diversity in this paper, we have classified the field experiments according to wheat sole crop performance as observed on control treatments without a cover crop. This classification relies on three indicators of wheat performance at harvest: aerial DM (kg ha^{-1}), total N content (kg ha^{-1}) and grain yield (dt ha^{-1}). We considered that the values of these indicators were the result of the environmental conditions observed on the eight experimental sites. Three groups of sites were determined according to the values of aerial DM, total N content and grain yield (Table 2).

2.5. Biomass and intercrop ratios

The aerial DM ratio of legume and wheat ($\mathrm{DM_L/DM_W}$) was chosen to describe legume growth compared to wheat at both wheat flowering stage and harvest time. An intercrop ratio, representing the value of relay intercropped treatment compared to the sole crop treatment, was also calculated to evaluate the effect of the RIL system compared to wheat sole crop. This intercrop ratio will be referred to as "L/T0" and placed after the name of the chosen indicator (grain yield, grain protein content [GPC], soil moisture, PAR*i* or wheat total N content) in the following sections. These ratios allow data with a high inter-site variability to be compared on the same basis.

2.6. Statistical analysis

Square root or log transformation were used on data when Levene's homogeneity of variance test ($P = 0.05$) was not significant. Two-factor ANOVAs were performed using XLStat software (Addinsoft SARL, Paris, France), with repeated measures in randomized complete blocks, to detect the effects and interactions of site, block and treatment. If a significant F test ($P = 0.10$) was obtained from ANOVAs, Tukey's HSD test was then used to determine treatment differences at $P = 0.10$. If the homogeneity of variance was not respected after data transformation, a non-parametrical test of Kruskal–Wallis (K–W, $P = 0.10$) was performed. Means were compared using a Dunn test when the K-W test was significant.

3. Results

3.1. Site grouping

On the basis of sole crop performance, three groups were defined, with low, intermediate and high wheat performance. The different thresholds for each wheat performance indicator are given in Table 2. A09, C09 and F10 were characterized by a combination of low wheat performance, E10 and G10 by intermediate wheat performance and B09, D09 and H10 by high wheat performance.

3.2. Legumes aerial biomass

At the wheat flowering stage, relay intercropped legumes showed variable aerial biomass ranging from 0 to 532 kg DM ha^{-1} (data not shown). This corresponded to a range of legumes/wheat aerial biomass ratios between less than 1% and 12%, with a mean of less than 4%. The growth of cover crops at the wheat flowering stage was limited and not linked to wheat performance and resource availability (data not shown). We have chosen in this paper to put the emphasis on data obtained at wheat harvest when the total effect of RIL system could be analyzed.

The four relay intercropped legume species presented highly variable aerial dry matter at wheat harvest ranging from 2 to 2880 kg DM ha^{-1} (Table 3a). The ranking order of the four species was very similar whatever the site. On average, *M. lupulina* and *T. pratense* exhibited the highest aerial dry matter (1371 and 1294 kg DM ha^{-1}, respectively), followed by *M. sativa* with 898 kg DM ha^{-1} and finally *T. repens* with 677 kg DM ha^{-1}. The site had a highly significant effect on results ($P < 0.001$).

At harvest, the ratio of legume/wheat aerial dry matter averaged 18% with a range varying from less than 1%–59% (Table 3b). On average, the ranking of treatments according to biomass ratios was the same as for respective values of raw legume aerial dry matter: *M. lupulina* and *T. pratense* represented the highest part of wheat aerial DM (22%), while *M. sativa* and *T. repens* represented respectively 16 and 11% of wheat aerial DM on average.

Legume growth was different in the three groups of fields determined from wheat sole crop performance (Table 3). Legume growth was significantly lower on sites where wheat sole crop performance was high than in the two other wheat performance groups ($P < 0.001$; data not shown). Inversely, legume growth was significantly higher for the intermediate wheat sole crop performance group than in the two other wheat performance groups. Finally, legume growth was highly variable depending on the site in the low wheat sole crop performance group but, on average, the mean aerial growth of the four relay intercropped legume species in this group was intermediate and significantly different from the two other groups. Concerning the year, in the two extreme (high and low) wheat sole crop performance groups, legume growth was higher in 2010 than in 2009 ($P < 0.001$; data not shown).

The ranking of the four relay intercropped legume species in each group of wheat sole crop performance was more or less the same and similar to the global ranking of the legume species with *M. lupulina* and *T. pratense* showing the best aerial growth followed by *M. sativa* and finally *T. repens*.

3.3. Effects of legumes on wheat performance

3.3.1. Grain yield

Sole crop winter wheat (T0) yielded from 21.6 to 44.7 dt ha^{-1} according to the sites, with a mean of 31.4 dt ha^{-1} (Table 4a). On relay intercropped treatments, grain yields ranged from 20.8 to 47.1 dt ha^{-1}, with an average of 30.3–31.2 dt ha^{-1} depending on legume species. The presence of legumes had, in general, no

Table 2
Site grouping according to the final performance of winter wheat sole crop: aerial dry matter (DM, kg ha^{-1}), total N uptake (kg ha^{-1}) and grain yield (dt ha^{-1}) at wheat harvest.

Wheat sole crop performance groups	Aerial DM (kg ha^{-1})	Total N uptake (kg ha^{-1})	Grain yield (dt ha^{-1})	Site codes
Low	<5500	<65	<25	A09 C09 F10
Intermediate	5500 < X < 7500	65 < X < 90	25 < X < 35	E10 G10
High	>7500	>90	>35	B09 D09 H10

Table 3
Aerial dry matter (DM) of relay intercropped legumes (kg ha^{-1}) (a) and ratios of aerial DMs of relay intercropped legumes and wheat (DM_L/DM_W, in %) (b) at wheat harvest for the sites grouped according to winter wheat sole crop final performance.

Groups of sites [a]	Low				Intermediate			High				Mean
Treatment	A09	C09	F10	Group mean	E10	G10	Group mean	B09	D09	H10	Group mean	
(a) Aerial DM of legumes												
M. lupulina	386 a	1627 a	2566	1526 a	2493 a	2072 a	2283 a	287 a	157	1376 a	607 a	1371 a
M. sativa	159 a	766 ab	2555	1160 ab	1410 bc	1238 b	1324 b	29 b	100	920 b	350 bc	898 bc
T. pratense	28 b	1365 a	2880	1424 ab	2302 ab	2203 a	2253 a	137 a	121	1318 a	525 ab	1294 ab
T. repens	2 c	301 b	2250	851 b	1248 c	1163 b	1205 b	4 c	48	266 c	106 c	677 c
Treatment *P*-value	< 0.001	< 0.001	0.17	0.036	< 0.001	< 0.001	< 0.001	< 0.001	0.16	< 0.001	< 0.001	< 0.001
Site effect ($P < 0.001$)	c	b	a		ab	ab		c	c	b		
(b) DM_L/DM_W												
M. lupulina	10 a	31 a	45 ab	29 a	40 a	32 a	36 a	6 a	2	13 a	23 a	22 a
M. sativa	4 ab	15 b	54 ab	24 ab	23 b	18 b	20 b	<1 bc	2	9 a	14 b	16 bc
T. pratense	1 bc	26 ab	59 a	29 ab	40 a	33 a	36 a	2 ab	2	13 a	19 a	22 ab
T. repens	<1 c	6 c	38 b	15 b	22 b	20 b	21 b	<1 c	1	3 b	7 c	11 c
Treatment *P*-value	<0.001	<0.001	0.03	0.024	<0.001	0.01	<0.001	<0.001	0.45	<0.001	<0.001	<0.001
Site effect ($P < 0.001$)	d	bc	a		ab	ab		d	d	c		

Letters in column indicate significant mean differences between treatments for each site and on average, according to Tuckey's HSD test or Dunn's test ($P = 0.10$). Letters in the last line of each sub-table indicate significant differences between sites, according to the same statistical tests. Framed cells indicate the relay intercropped treatments where grain yield was significantly reduced compared to the control treatment. Dark cells indicate the relay intercropped treatments where GPC was significantly reduced.

[a] According to final performance of wheat sole crop (Table 2).

significant effect on wheat grain yield. A significant reduction of wheat grain yield on relay intercropped treatments was only observed on one out of thirty-two treatments with *T. repens* at E10 (−22% compared with T0). No difference amongst the type of legumes was observed on six out of eight sites. On site E10, wheat relay intercropped with *T. repens* obtained a significantly lower yield compared to *M. lupulina* and *M. sativa* treatments. On site F10, *T. pratense* obtained a significantly lower yield compared to *M. lupulina* and *T. repens*. The effect of the site on wheat grain yield was highly significant ($P < 0.001$; Table 4a). The ranking of the sites was similar to wheat sole crop performance groups' one (Table 2) with B09, D09 and H10 of the high wheat sole crop performance group showing the best average wheat grain yields compared to the other sites and A09, C09 and F10 of the low wheat sole crop performance group showing the lowest average wheat grain yields.

Table 4
Grain yield of winter wheat at 15% of moisture content (dt ha^{-1}) (a) and wheat grain protein content (GPC, %DM) (b) for the sites grouped according to winter wheat sole crop final performance.

Groups of sites [a]	Low				Intermediate			High				
Treatment	A09	C09	F10	Group mean	E10	G10	Group mean	B09	D09	H10	Group mean	Mean
(a) Grain yield												
Control	21.6	23.4	23.7 ab	22.9	31.1 a	29.6	30.4 a	36.0	41.3	44.7	40.7	31.4
M. lupulina	21.9	25.3	25.3 a	24.2	29.5 a	26.5	28.0 ab	28.3	39.4	45.9	37.9	30.3
M. sativa	23.1	25.8	22.4 ab	23.8	29.5 a	28.9	29.2 a	33.0	41.0	44.5	39.5	31.0
T. pratense	25.4	25.9	21.1 b	24.1	29.4 ab	27.8	28.6 a	32.5	43.1	44.4	40	31.2
T. repens	20.8	27.1	25.4 a	24.4	24.2 b	25.8	25 b	33.6	40.2	47.1	40.3	30.5
Treatment *P*-value	0.37	0.43	0.06	0.76	0.008	0.11	0.003	0.14	0.86	0.88	0.62	0.92
Site effect ($P < 0.001$)	d	cd	d		bc	bc		b	a	a		
(b) GPC												
Control	9.3	10.1 a	10.3 a	9.9 a	10.5 a	10.5 ab	10.5 a	10.1	9.4	10.2	9.9	10.0
M. lupulina	9.7	9.5 b	9.2 b	9.5 b	9.3 c	10.2 bc	9.8 c	10.3	9.2	10.4	10.0	9.7
M. sativa	9.2	10.1 a	9.6 ab	9.6 ab	9.7 bc	10.4 ab	10.1 b	9.8	8.4	9.8	9.3	9.6
T. pratense	9.4	9.8 ab	9.2 b	9.5 b	9.4 bc	10.0 c	9.7 c	10.1	9.8	10.6	10.2	9.7
T. repens	9.3	10.2 a	9.5 b	9.7 ab	9.8 b	10.7 a	10.3 ab	10.8	8.6	10.0	9.8	9.9
Treatment *P*-value	0.12	0.022	0.006	0.007	0.001	0.005	<0.001	0.23	0.39	0.75	0.21	0.28
Site effect ($P < 0.001$)	cd	abc	bcd		abcd	a		ab	d	ab		

Letters in column indicate significant mean differences between treatments for each site and on average, according to Tuckey's HSD test or Dunn's test ($P = 0.10$). Letters indicate significant differences between sites, according to the same statistical tests. Framed cell indicates the relay intercropped treatments where grain yield was significantly reduced compared to the control treatment. Dark cells indicate the relay intercropped treatments where GPC was significantly reduced.

[a] According to final performance of wheat sole crop (Table 2).

3.3.2. Wheat grain protein content

On the control treatment (T0), wheat GPC ranged from 9.3% to 10.5% DM with a mean of 10.0% DM (Table 4b). When relay intercropped, wheat GPC varied from 8.4% to 10.8% DM with a mean ranging from 9.65% to 9.9% DM depending on the legume species. The total GPC of relay intercropped wheat was significantly reduced by relay intercropped legumes in nine out of thirty-two treatments. The degree of reduction varied from 5% to 11% of the sole crop treatment value. The effect of the site was highly significant ($P < 0.001$; Table 4b) but the differences between sites were not statistically marked and were not particularly associated with the grouping of sites based on the three indicators of the Table 2.

Treatments relay intercropped with *M. lupulina* and *T. pratense* were especially characterized by low wheat GPC. These two species of legume make up two thirds of the depleted treatments. Six out of the nine depleted treatments were relay intercropped with these two species. On the remaining treatments (23 out of 32), no significant effect of the presence of relay intercropped legumes was observed.

3.4. Legume growth and wheat performance

The nine treatments where grain yield and/or GPC were limited by RIL (*M. lupulina*: C09, E10 and F10; *T. pratense*: E10, F10 and G10; *T. repens*: E10 and F10; *M. sativa*: E10; Table 4) were also the treatments where these legumes had the highest growth compared to wheat. At wheat harvest, we observed that 9 out of 12 treatments showing DM_L/DM_W ratios higher than 20% were associated with significant wheat grain yield and/or GPC reduction (framed and darkened cells in Table 3b). Then we considered this ratio of aerial biomasses as a threshold upon which the rest of our analysis concerning the links between legumes growth and wheat performance on the one hand and resource availability on the other hand was developed.

This threshold ratio appeared to be adapted when considering wheat GPC. Below the DM_L/DM_W ratio threshold of 20%, the intercrop ratio (L/T0) for GPC has exhibited high variability around one without any significant limitation of GPC (Fig. 1b). Above this ratio, all GPCs of relay intercropped treatments were below GPCs of sole crop treatments, indicating depletion of the GPC on corresponding relay intercropped treatments. In 9 out of the 12 cases below one, the GPC was significantly decreased.

Concerning wheat grain yield, intercrop ratios showed great variability, from 0.78 to 1.18. (Fig. 1a). Approximately one third of relay intercropped treatments exhibited wheat grain yield equal to or higher than what was obtained in the control treatment indicating an advantage of the RIL situation compared to wheat sole crop. Almost all treatments with equal or higher performance values with relay intercropped treatments came from the low wheat sole crop performance group (9 out of 12 treatments).The remaining two-thirds were beneath control treatment values and only the lowest value – see framed symbol – showed significant yield penalty. All relay intercropped treatments coming from the intermediate wheat sole crop performance group obtained lower values than sole crop.

3.5. Legume growth and resource availability

The growth of legumes depleted soil moisture in almost all situations (thirty out of thirty-two treatments) compared to control treatments (Fig. 2). On the sites registering high wheat sole crop performance, legume growth was low compared to wheat. Consequently, soil moisture depletion was limited and generally did not exceed by more than 10% the sole crop treatment value (9 out of 12 treatments). For the two remaining wheat sole crop performance groups, soil moisture variation of relay intercropped treatments

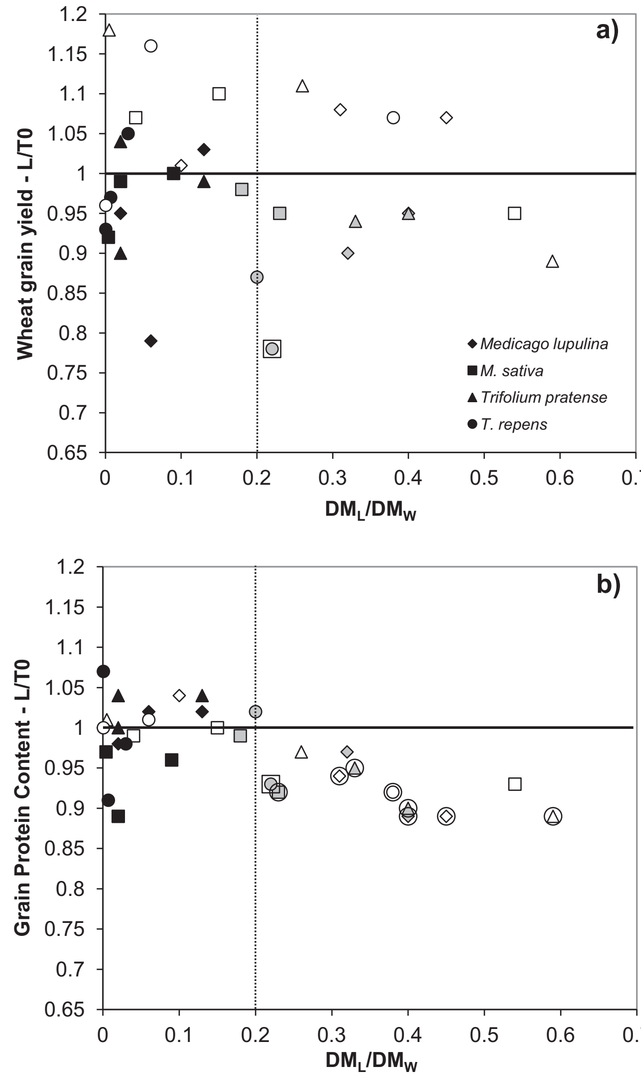

Fig. 1. Ratios of wheat grain yield (a) and grain protein content (b) expressing the values of relay intercropped wheat to wheat sole crop (L/T0) related to ratios of legume/wheat aerial biomass (DM_L/DM_W) at wheat maturity. The symbols correspond to the four relay intercropping treatments: *M. lupulina* (♦), *M. sativa* (■), *T. pratense* (▲) and *T. repens* (●). The color of the symbol indicates the level of final performance of wheat sole crop. Black points correspond to sites where final performance of winter wheat were high, gray points to intermediate and white points to low (see Table 2). The vertical dotted line represents the 20% threshold of DM_L/DM_W. The framed symbol (□) corresponds to the treatment where a significant decrease in wheat grain yield and protein content were observed. Circled symbols (◯) correspond to treatments where significant decreases in wheat grain protein content were observed.

compared to sole crop were highly variable (Fig. 2) and the majority of the treatments exhibited soil moisture depletion more than 10% higher than the sole crop treatment values. Treatments where wheat GPC or grain yield were significantly limited obtained levels of soil moisture depletion equal to or higher than 10% compared to sole crop treatments, reaching a maximum of 33% less soil moisture than in sole crop treatment.

Concerning relay intercropped wheat N uptake, the responses to the RIL situation was clearly different from one performance group to another (Fig. 3). We first observed that, in general, DM_L/DM_W ratios over 20% were associated with lower wheat N uptake than those observed for low biomass ratios (Fig. 3). Then, total N content of relay intercropped wheat at harvest was systematically limited on sites with intermediate wheat sole crop performance. On the contrary, total N content of relay intercropped wheat at harvest

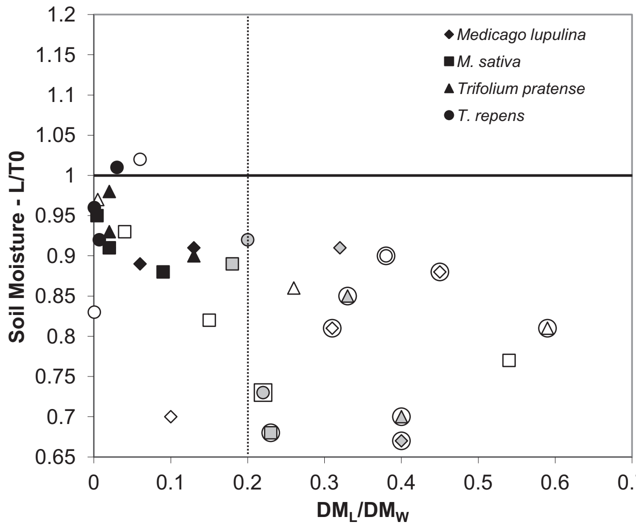

Fig. 2. Ratios of soil moisture expressing the values of relay intercropped treatments to wheat sole crop treatment (L/T0) related to legume/wheat aerial biomass ratios (DM_L/DM_W) at wheat maturity. The symbols correspond to the four relay intercropping treatments: *M. lupulina* (♦), *M. sativa* (■), *T. pratense* (▲) and *T. repens* (●). The color of the symbol indicates the level of final performance of wheat sole crop. Black points correspond to sites where final performance of winter wheat were high, gray points to intermediate and white points to low (see Table 2). The vertical dotted line represents the 20% threshold of DM_L/DM_W. The framed symbol (□) corresponds to the treatment where a significant decrease in wheat grain yield and protein content were observed. Circled symbols (◯) correspond to treatments where significant decreases in wheat grain protein content were observed.

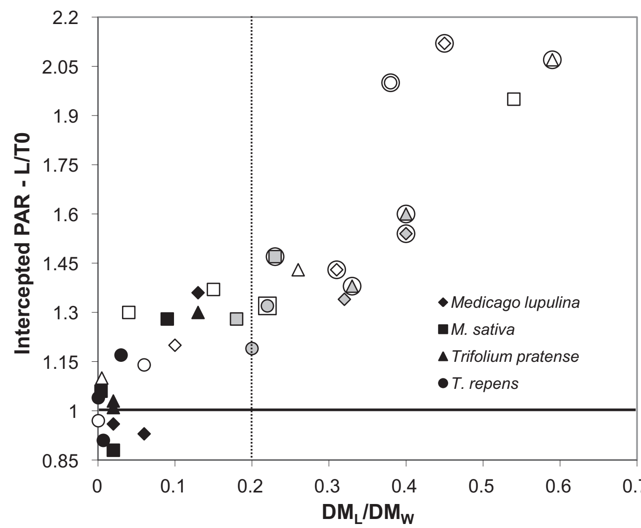

Fig. 4. Ratios of total incident photosynthetically active radiation (PAR) intercepted by whole field cover expressing the values of relay intercropped treatments to wheat sole crop treatment (L/T0) related to legume/wheat aerial biomass ratios (DM_L/DM_W) at wheat maturity. The symbols correspond to the four relay intercropping treatments: *M. lupulina* (♦), *M. sativa* (■), *T. pratense* (▲) and *T. repens* (●). The color of the symbol indicates the level of final performance of wheat sole crop. Black points correspond to sites where final performance of winter wheat were high, gray points to intermediate and white points to low (see Table 2). The vertical dotted line represents the 20% threshold of DM_L/DM_W. The framed symbol (□) corresponds to the treatment where a significant decrease in wheat grain yield and protein content were observed. Circled symbols (◯) correspond to treatments where significant decreases in wheat grain protein content were observed.

was improved on sites with low wheat sole crop performance in 7 out of 12 treatments. Total N content of relay intercropped wheat had decreased in 8 out of 12 treatments on sites with high wheat sole crop performance. Decreases in N uptake of relay intercropped wheat compared to wheat sole crop were linked to significant decreases in GPC on eight out of nine treatments.

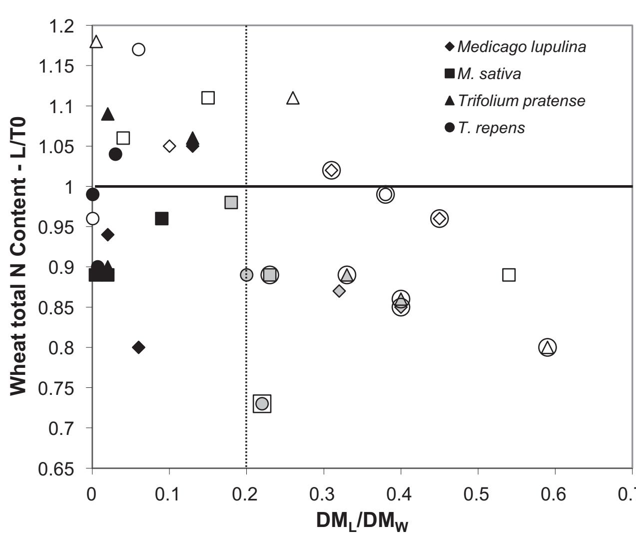

Fig. 3. Ratios of wheat N content expressing the values of relay intercropped wheat to wheat sole crop (L/T0) related to legume/wheat aerial biomass ratios (DM_L/DM_W) at wheat maturity. The symbols correspond to the four relay intercropping treatments: *M. lupulina* (♦), *M. sativa* (■), *T. pratense* (▲) and *T. repens* (●). The color of the symbol indicates the level of final performance of wheat sole crop. Black points correspond to sites where final performance of winter wheat were high, gray points to intermediate and white points to low (see Table 2). The vertical dotted line represents the 20% threshold of DM_L/DM_W. The framed symbol (□) corresponds to the treatment where a significant decrease in wheat grain yield and protein content were observed. Circled symbols (◯) correspond to treatments where significant decreases in wheat grain protein content were observed.

The proportion of intercepted PAR was higher when wheat was relay intercropped with legumes than for wheat sole crop in almost all situations, and increased with legumes relative growth (Fig. 4). The relationship between the two variables was clearly significant ($P < 0.001$, $R^2 = 0.82$). On sites where wheat sole crop obtained high performance levels, the limited growth of legumes induced limited increases in intercepted light. The proportion of intercepted PAR for the two other performance groups were less distinct. High DM_L/DM_W ratios over 20% were associated with proportions of intercepted PAR by the whole cover at wheat harvest more than 30% higher than the wheat sole crop values (Fig. 4). The nine treatments where wheat grain yield and/or GPCs were decreased by RIL showed PAR interception gains ranging from 32% to 112% of the sole crop treatment values.

4. Discussion

4.1. Effects of RIL on wheat performance

4.1.1. Wheat grain yield

The range of sole crop grain yield was similar to grain yields previously observed in organic unfertilized conditions in south-east France (David et al., 2005a,b).

Under organic conditions with no additional fertilization, RIL did not induce a significant reduction in grain yield, except on one out of thirty-two treatments. These results were obtained under variable soil and weather conditions, and clearly demonstrate that organic wheat grain yield is not affected even though the four legumes species showed various degrees of aerial growth (Table 3). This result is consistent with previous studies on RIL with the same species in conventional (Thiessen Martens et al., 2001; Mutch et al., 2003; Blackshaw et al., 2010; Bergkvist et al., 2011) and organic agriculture (Hartl, 1989; Becker and Leithold, 2008; Olesen et al., 2009).

4.1.2. Grain protein content

Low values of GPC of around 10% DM observed on control treatments are consistent with previous data obtained under similar organic conditions (David et al., 2005a; Casagrande et al., 2009).

In this study, wheat GPC of relay intercropped treatments decreased in 28% of cases tested, even in those cases where the values were already low in sole crop treatments. This was especially true for wheat relay intercropped with *M. lupulina* and *T. pratense* which developed the highest aerial DMs after flowering.

The few studies interested in the effect of RIL on wheat GPC found results contrary to ours with no effect of relay intercropped red clover or alfalfa (Blackshaw et al., 2010) or a mixture of legumes cover crops (Becker and Leithold, 2008) on winter wheat quality.

We hypothesized that the delay between the sowing dates of the relay intercropped species was supposed to avoid too intense competitive relationship between them leading to decreased wheat performance. The final performance of relay intercropped wheat that we observed show that the implementation of the RIL technique in organic winter wheat can lead to decreased wheat performance, and especially wheat grain quality when the conditions are favorable to relay intercropped legumes.

4.2. Effects of RIL on resource availability

4.2.1. Soil water content

Our experiments showed that the relay intercropped legumes directly affect water availability in the first 90 cm of soil during the crop cycle. Adding a second crop to wheat led logically to greater soil water use through increased canopy transpiration from cover and cash crops (Thorsted et al., 2006). Field experiments characterized by high wheat sole crop performance were less impacted by limited water availability due to the low growth of legumes. Furthermore, our results have shown variability in the effect the four species have had on water availability. For instance, *M. sativa*, which has a strong tap root system (Frame, 2005) depleted soil moisture more than *T. pratense* despite lower aerial DM.

Concerning the rooting behavior of the relay intercropped species we can assume that, in the RIL system, the time between the two sowing dates allowed wheat roots to reach deeper water reserves before the legumes were sown. Moreover, topsoil water competition from growing legumes may have led wheat roots to grow to a greater depth (Hauggaard-Nielsen and Jensen, 2005; Li et al., 2011). Therefore, the drying up of the topsoil, aggravated by undersown legumes, may not have disturbed wheat water nutrition if the rooting system were not limited by soil compaction. For instance, Shili-Touzi et al. (2010) and Carof et al. (2007b), analyzing the rooting system of intercrops, showed that the intercropping technique did not affect soil water availability to the cash crop.

Beyond the fact that water is a trophic resource for plants *per se*, its availability could directly affect N mineralization from soil leading to a potential influence on post-flowering N nutrition during the grain filling period (Paul et al., 2003).

4.2.2. Wheat N uptake

Under limited N availability conditions, legumes are supposed to rely on symbiotic fixation for their N nutrition, limiting interspecific competition with wheat for soil mineral N through complementary N resource use (Corre-Hellou et al., 2006). Nevertheless, in this study, high legume DM biomass was associated with limited total N uptake and wheat GPC at harvest time, indicating that GPC decline at the expense of legume growth.

In organic grain systems, N availability for winter wheat may be limited in cases where no additional fertilization was added. N availability depends exclusively on crop residues and mineralization of soil organic matter to ensure wheat's N demand. The reduction in soil water availability observed in relay intercropped treatments may have led to limitations in biological activity and soil organic matter mineralization (Paul et al., 2003; Celette et al., 2009). The colonization of the topsoil layer by both wheat and emerging legume roots may lead to competition for water and mineral resources. The topsoil layer is the predominant soil N reserve as crop residues and amendments are incorporated in this layer. As a consequence of the top soil drying up, it is likely that residual mineral N or mineralized organic N becomes immobilized and unavailable to wheat.

4.2.3. Intercepted radiation

Our results showed a positive relationship between light interception by the whole canopy and the DM_L/DM_W ratio at wheat harvest. Potential competition for light should occur if legumes are sufficiently developed at an early growing stage of wheat and their canopy reaches that of the wheat leaf (Carof et al., 2007b). However, in the early stages of RIL, the dominance of wheat over legumes implies that light competition was not to the detriment of wheat but to the detriment of legumes (Queen et al., 2009; Shili-Touzi et al., 2010). During grain filling, the senescence of older wheat leaves may have permitted an increase in light transmittance to the cover crops (Blaser et al., 2006). Legume development between wheat flowering and wheat harvest increased rapidly due to this rise in light transmittance. Thus, the shading effect of legumes over wheat will probably only have occurred in the highest legume species, i.e. *M. sativa* and to a lesser extent *T. pratense*, for a few days before wheat harvest.

Due to its erect growth (Frame, 2005), *M. sativa* should have been wheat's strongest light competitor. However, this species did not intercept more light than other species at wheat harvest time due to lower aerial DMs in our experiment (Table 3b) (Thiessen Martens et al., 2001).

Lower, but denser canopies of *M. lupulina* and *T. pratense* did not present this drawback and stayed far below the level of the spikes. The prostrate growth pattern of *T. repens*, in addition to poor aerial development, offered little threat to wheat for light sharing.

The increase in radiation interception of relay intercropped treatments with a higher legume biomass in the whole canopy, indicates better use of solar radiation while limiting the amount of light that reached the ground. The poor development of legume cover crops on fields with high wheat sole crop performance limited the radiation use and its potential benefits.

A reduction in the amount of light reaching the ground, courtesy of the cover crops, is expected to be beneficial to weed control (Kruidhof et al., 2008). Thus, the denser legume canopies, represented by *M. lupulina* and *T. pratense* can be expected to provide better weed control after harvest than other species.

4.3. Effects of legumes on wheat performance through resource availability

The relation between resource availability in a RIL system and its effect on cash crop performance has not been addressed in previous papers. The monitoring of legumes' growth and resources availability has enabled us to define ranges of values above which limitations in wheat GPC and yield may appear.

Under the conditions explored in our field experiments, there was a significant decrease in the final outcomes for wheat and especially in GPC on relay intercropped treatments for ratios of legume/wheat biomass higher than 20% at wheat harvest (Table 3b). Such a ratio, equal to or higher than 20%, which corresponds to the lowest DM ratio above which final wheat performance were significantly depleted, was obtained equally as well with a high growth of legumes in a wheat crop showing intermediate performance (site E10 and G10) as with a high legume development in poorly developed wheat (site C09 and F10).

The decline in performance was related to combined disturbances to the availability of two main trophic resources, which are soil water and soil N. In fact, relay intercropped treatments in which wheat performance was penalized showed significantly lower soil water content ($P < 0.001$) ranging from −10% to −33% of the control treatment value with an average of −22%. Similarly, wheat N uptake was also significantly lower in these penalized treatments ($P = 0.034$) with variations ranging from +2% to −27% of the control treatment value with an average of −11%.

4.4. The establishment of legume cover crops

We expected that, despite the delay in the sowing dates of wheat and legume cover crops, the environmental conditions surrounding legumes (pedo-climatic conditions and wheat competition) would be favorable in early spring to allow their establishment and growth in wheat. Our results show that it is dependent both on legume species and on the wheat final performance potential of each field.

M. lupulina and *T. pratense* withstand the competition of wheat the best during their establishment in spring and early summer. In conventional RIL conditions, *T. pratense* was also found to develop higher aerial biomass than *T. repens* (Bergkvist et al., 2011) and *M. sativa* (Thiessen Martens et al., 2001; Blaser et al., 2011). Our results also showed that *M. sativa*, despite having an erect growth pattern, did not benefit from this characteristic when relay intercropped with wheat, since its aerial development represented, on average, only 65% of *M. lupulina*'s which grows prostrate. *T. repens* is the legume that showed the least development in the RIL system, whatever the year and the site. Its naturally slow growth (Frame, 2005) was confirmed here and aggravated by competition with wheat.

Our experiment showed that on sites where wheat performed well (Table 2), legume development was low relative to wheat. This situation was associated with limited or no variations in wheat performance or resource availability compared to wheat sole crop. The poor establishment of legumes on the field, as shown in conventional conditions by Blaser et al. (2011) after highly competitive wheat cultivars, may lead to limited agronomic efficiency in facing soil N deficiencies (Bergkvist et al., 2011) and weed infestation after wheat harvest.

On the contrary, on sites where wheat performed poorly (Table 2), legume development relative to wheat was highly variable. Even though the aerial development of wheat was potentially low in this group of sites, thereby reducing its competitiveness, the establishment of legumes under wheat may still be limited when environmental conditions are unfavorable (e.g. stony soil and low temperatures on site A09). On the other hand, if the conditions for the cover crop are good, it may develop very strongly due to the low competitiveness of the cereal (e.g. deep loamy soil, wet and hot weather conditions on site F10). In terms of interactions for trophic resources, it appeared that even with low or intermediate development of cover crops, when wheat performance were low, relay intercropped wheat grain yield and N content at harvest were higher than values for wheat sole crop (Fig. 1a and Fig. 3).

When sites were characterized by intermediate wheat performance (Table 2), the aerial development of legumes was also intermediate in the range of values observed in our experiment. These aerial developments were almost systematically associated with lower performance of relay intercropped wheat, soil water content or wheat N content compared to wheat sole crop. The competitiveness of wheat in these environmental conditions was not sufficient to overcome the capture of trophic resources by legumes, which led to significant decreases in performance of relay intercropped wheat.

Climatic conditions in 2010 were wetter than in 2009 (604 vs. 508 mm, respectively, on average from wheat sowing to harvest for all sites) but still hot (10.2 vs. 9.8 °C, respectively, on average from wheat sowing to harvest for all sites) (Table 1). Despite the fact that wheat also benefited from these hotter and more humid conditions (7116 vs. 8764 kg ha^{-1} of aerial DM in 2009 and 2010, on average, respectively) and should have competed stronger with legumes for light, these climatic conditions also benefited legume growth during the second year (345 vs. 1766 kg ha^{-1} of aerial DM in 2009 and 2010, respectively, on average for all species at wheat harvest time) leading to competition for trophic resources and cash crop performance penalties in some cases.

Legume species that exhibit good aerial development at wheat harvest time, especially *M. lupulina* and *T. pratense*, are assumed to play an important role in the agro-ecological improvement of the system through the (i) production of primary biomass, carbon sequestration and improvement of soil organic matter content; (ii) recycling of nutrient elements, especially N made available to the subsequent crop and (iii) interception of radiation that might otherwise reach weeds, and the limitation of soil erosion and crusting, all associated with ground covering (Sheaffer and Seguin, 2003; Shili-Touzi et al., 2010).

This study took place within an exploration of organic farming in which weeds are an important issue. As the ratios of weed aerial DMs compared to wheat on control treatments were considered as weak (2.7% on average), we decided not to integrate them in the analysis. Their role in the RIL system and the control played by cover crops should be considered in another study.

5. Conclusions

Although the aerial development of relay intercropped legumes did not lead to wheat grain yield penalties, whatever the conditions of climate, soil or wheat growth, wheat grain protein content was depleted in about one third of the cases. These penalized treatments exhibited high aerial development in the four legume species. *M. lupulina* and *T. pratense*, were the species which posed the greatest risk to wheat quality. *M. sativa* and *T. repens* suffered from wheat dominance and represented a lower risk to wheat performance.

The choice of legume cover crops for relay intercropping should take into account the potential of wheat performance in the field where RIL is planned to be implemented. The high competitiveness of a wheat crop with high performance potential led to limited cover crop establishment and limited competition. But poor cover crop development may limit their contribution to long-term agro-ecological interests.

Our experiments showed that establishing legume cover crops in organic winter wheat at the end of wheat tillering stage permits to reach high levels of biomass by harvest time, when the growing conditions are favorable. The agro-ecological interests associated with it are multiple and need further interest. This is true for light transformation and biomass production and also potential weed control as we showed that much more light was intercepted in the presence of legume cover crops compared to the pure wheat crop and light reaching the ground was thus highly diminished. The issue of weeds is particularly questioned in organic grain production. N accumulation in cover crop biomass, that is of great importance for the enrichment and preservation of N in the soil-plant system and the success of the subsequent crop, is also an important issue in organic grain production. As for the improvement of soil N content and weed control, *M. lupulina* and *T. pratense*, which developed the highest biomasses under wheat, are thought to be of greatest interest.

Acknowledgements

This work was partly financed by the CORE Organic ERA-net in the research project AGTEC-Org. We would like to thank the Jouffray Drillaud firm for providing legume seeds, T. Lhuillery and the interns for technical support and C. Holland for revising the text in English. Finally, we would like to give special thanks to the organic farmers (J.-M. Brun, M. Breyton, J.-M. Chancel, P. Robin, P. Vacher and D. Valentin) without whom this study would not have been possible.

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