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Specific interactions leading to transgressive overyielding in cover crop mixtures

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ABSTRACT

Growing mixtures of species instead of sole crops is expected to increase the ecosystem services provided by cover crops. This study aimed at understanding the interactions between species and investigating how they affect the performance of the mixture. Four species were combined in six bispecific mixtures in a field experiment. The performance of each species when grown in a mixture was compared to its performance as a sole crop at different sowing densities, to characterise the influence of intra- and interspecific competition for each species. Intra- and interspecific competition coefficients were quantified using a response surface design and the hyperbolic yield-density equation. Interactions between the four species ranged from facilitation to competition. Most of the mixtures exhibited transgressive overyielding. Without nitrogen (N) fertilisation, high complementarity between species allowed to achieve the highest biomass. With N fertilisation, high dominance of one mixture component should be avoided to achieve good performance. A revised approach in the use of the land equivalent ratio for the evaluation of cover crop mixtures is also proposed in this study. It allows to better identify transgressive overyielding in mixtures and to better characterise the effect of one species on the other within the mixture.

1. Introduction

Currently, there is growing interest in improving integration of cover crops in cropland. Cover crops are grown between two cash crops and can provide a wide range of ecosystem services such as weed control (Brust et al., 2014), soil protection against erosion (De Baets et al., 2011) or increase of soil organic matter (Ding et al., 2006). They are also able to recycle large amounts of nutrients and may thus prevent their losses (Thorup-Kristensen et al., 2003). Some species can mobilise poorly available nutrient forms (Hunter et al., 2014; Nuruzzaman et al., 2005) and legume species can symbiotically fix nitrogen (N) (Büchi et al., 2015). Cover crop cultivation also contributes to increasing diversity in cropland, which is paramount for the sustainability of agroecosystems (Altieri, 1999).

Growing mixtures of cover crop species instead of sole crops may improve the services offered, but also allow providing several services at the same time. Currently, most studies showing benefits of mixed cropping systems with cover crops involve associations of legume and non-legume species. It has been reported that these mixtures can simultaneously reduce nitrate leaching and fix N from the atmosphere (e.g. Tosti et al., 2014; Tribouillois et al., 2015). Tosti et al. (2014) showed that the carbon to nitrogen (C/N) ratio of the mixture barley-vetch should allow faster mineralisation than that of barley as a sole crop. Other services, such as a higher weed suppression by the mixture compared to legume alone (Akemo et al., 2000), have been reported. Contrary to mixtures of legume and non-legume species, associations of two non-legume species are rare (e.g. Finney et al., 2016). These mixtures may however be of interest for diversifying the ecosystem services, such as weed control and nutrient recycling, especially when N is not limiting.

Most of the services provided by cover crops are intimately related to the biomass productivity of the crops (Finney et al., 2016). Maximising biomass production is thus an interesting way to optimise the services provided by a cover crop. This could be

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achieved for mixtures exhibiting ‘overyielding’ or, even better, ‘transgressive overyielding’ (Sainju et al., 2006; Schmid et al., 2008; Wang et al., 2012). Overyielding occurs in a mixture ‘when its biomass production is greater than that of the average monoculture of the species contained in the mixture’ (Schmid et al., 2008). Transgressive overyielding appears ‘when the productivity in mixture is larger than the maximal productivity of the constituent species’ (Gravel et al., 2012). Transgressive overyielding should thus be the main goal when associating cover crop species.

However, to improve biomass production of mixtures, a better understanding of how species interact is essential. Studies on species interactions in cover crop mixtures are limited but intercropping systems, where two or more crops are growing simultaneously in the same field, can be used as references (Vandermeer, 1989; Bedoussac et al., 2015; Brooker et al., 2015; Yu et al., 2015). Several types of interactions can be observed, ranging from negative effects due to competition to positive effects through facilitation. Facilitation, i.e. positive influence of the associated species, is of crucial importance as it leads to a better performance of the focal species in mixture than in sole crop (Brooker et al., 2016). For example, Maltais-Landry (2015) found that cereals in mixtures with legumes produce a greater biomass, and achieve a higher phosphorus (P) uptake and N concentration, than as sole crops. Facilitation can result from several mechanisms such as an increased resource availability (Zhang and Li, 2003; Li et al., 2014), or a decrease in disease and pest attacks (Hauggaard-Nielsen et al., 2008).

By contrast, some species may have a negative influence on the other species, resulting in a decreased performance in mixture compared to the sole crop. Negative influence may arise from competition for a limiting resource, such as nutrients, water or light, or from modification of the growing conditions (interference), for example allelopathic effects (Vandermeer, 1989).

In some cases, species may not influence each other. This situation can be observed when species use complementary resources and thus do not compete for the same resources. This leads to a more efficient resource capture of the mixture compared to sole crops. Complementarity in the use of N sources has been largely evidenced for associations of legume and non-legume species (e.g. Hauggaard-Nielsen et al., 2001a; Bedoussac and Justes, 2010; Cong et al., 2015; Li et al., 2016). In these associations, legume species increase their reliance on atmospheric N as the non-legume species are more competitive for soil N. Hauggaard-Nielsen et al. (2001b) showed that complementarity can also occur along the soil profile, between species exhibiting complementary rooting depths, such as pea and barley, leading to a better soil exploration.

Interactions between species are complex and influenced by many factors like nutrient availability, mixture density and the relative proportion of species (Connolly et al., 1990). In the case of mixtures combining legume and non-legume species, it has been shown that N fertilisation favours mainly the non-legume species at the expense of the legume, while at low N availability, the contrary was observed (Möller et al., 2008). Changes in species proportion and sowing density can also modify species relative competitive strength (Hauggaard-Nielsen et al., 2006).

In order to get the highest benefits from mixtures, it is thus essential to understand the influence of the following factors on mixture performance: relative proportion of species, sowing densities and fertilisation levels. It is also important to characterise and quantify intra- and interspecific interactions. To investigate species interactions in bispecific mixtures, Inouye (2001) suggested using a response surface design in which the density of each species is varied independently. This design allows distinguishing the effects of intra- and interspecific competition, through the coefficients of the yield-density equation (Wright, 1981). The benefit of mixtures over sole crops (e.g. transgressive overyielding) and species influence on the performance of the associated species can also be assessed with this design. The strength of the response surface design is to allow a global assessment of mixture performance, while more simple designs, such as replacement series coupled to commonly used indices such as land equivalency ratio (LER), only quantify the performance of each single mixture separately.

In this study, in order to understand interactions in bispecific mixtures, a response surface design involving four contrasting species, commonly used as cover crops under European conditions, was set up. The chosen species were: Indian mustard (*Brassica juncea*), field pea (*Pisum sativum*), black oat (*Avena strigosa*) and phacelia (*Phacelia tanacetifolia*). Mustard is expected to be a highly competitive species due to its allelopathic potential, while pea could have a positive influence on the associated species as it is able to biologically fix N. Oat and phacelia should have more neutral effects.

The main objectives of this study were (i) to evaluate the potential advantage of bispecific mixtures over sole crops, (ii) to determine the influence of each species on the performance of the associated species and (iii) to investigate how the interactions between species influence the performance of the mixture. All these factors were studied with and without N fertilisation to assess the importance of nutrient availability in driving species interactions and mixture performance.

2. Materials and methods

2.1. Site description and experimental design

The study was conducted in 2014 at Agroscope Changins (46°23′44.6″N, 6°14′24.6″E, 426 m asl), Switzerland, on a Cambisol (FAO classification system) with 244 g/kg of clay and 294 g/kg of sand in the top 20-cm soil layer. The average total annual precipitation is 999 mm and the mean temperature 10.2 °C (30-year averages, 1981–2010). Mineral N after cover crop emergence was 27 kg/ha for the 0–30 cm layer, 34 kg/ha for 30–60 cm and 24 kg/ha for 60–90 cm. Two field experiments were carried out with four species commonly used as cover crops under Swiss conditions: Indian mustard (*B. juncea* cv Vitasso), field pea (*P. sativum* cv Arkta), black oat (*A. strigosa* cv Pratex) and phacelia (*P. tanacetifolia* cv Balo).

In the main experiment, each species was grown in bispecific mixtures in combination with each of the other species, resulting in six different mixtures. For each mixture, twenty variants were studied: five different ‘relative proportions’ of the two species (0:1, 1:3, 1:1, 3:1 and 1:0) and four ‘mixture sowing densities’ (50%, 75%, 100% and 125%) (Fig. S1, Supporting Information). The sowing density of each species was thus the product of species relative proportion and mixture sowing density. The experiment was conducted with two nitrogen (N) fertilisation levels: 0 kg/ha and 30 kg/ha, applied as ammonium nitrate 12 days after sowing. The design of this experiment corresponded to a response surface design (Inouye, 2001) and included 176 plots (no replicates).

The main experiment was complemented by another experiment, set up on the same date and in the same field. In this experiment, each species was sown as a sole crop at six different sowing densities: 10%, 20%, 35%, 50%, 75% and 100% of their standard sowing density (see below). The experimental design followed a randomised block design with four replicates. 30 kg/ha of N were applied to the whole experimental field 12 days after sowing. This complementary experiment was used to assess the influence of sowing density on the biomass production of sole crops and thus allowed to characterise intraspecific competition. It was also used as reference to distinguish the effects of interspecific competition of different associated species from intraspecific effects.

For both experiments, the standard targeted sowing densities (100%) were 500 pl/m^2 for mustard and phacelia, 150 pl/m^2 for pea and 400 pl/m^2 for oat. The seeds were sown in 10 m^2 plots with an experimental seeder with 13.5 cm row spacing at 2 cm depth within the same row, on 6 August 2014 following winter wheat (straw exported, harrowed).

2.2. Measurements and analyses

Plant emergence was assessed by counting the seedlings about 20 days after sowing, in the sole crops and in the mixtures with equal relative proportion of each species (1:1). The effective plant density was then extrapolated for each sowing density, using the linear regression of the observed emergence counts on the theoretical density.

For the sole crops, at standard sowing density, the percentage of soil cover by the cover crops was assessed visually 13, 17, 19, 21, 23, 27, 29, 33, 40, 48, 57 and 65 days after sowing. Species initial growth rate was then estimated as the percentage of soil cover at 20 days after sowing. It was assessed by a Gompertz function (Bodner et al., 2010) fitted on the soil cover observations.

Shoot biomass production was measured 82 days after sowing in the main experiment (532 growing degree days (GDD) $T_{\text{base}} = 10\,^{\circ}\text{C}$) and 69 days after sowing (488 GDD) in the complementary experiment by the harvest of the above-ground parts at the ground level from 0.5 m^2 per plot. For mixtures, biomass production of each species was assessed separately. The samples were dried for 72 h at 55 °C, weighed and analysed for N concentration by near-infrared spectroscopy using a NIRS6500 (FOSS NIRSystems, Inc., Laurel, MD, USA).

2.3. Data analysis

2.3.1. Performance of mixtures and sole crops

For each mixture, the effect of sowing density and of species relative proportion on biomass production was assessed by a multiple linear regression.

To evaluate the performance of mixtures compared to sole crops, polynomial regressions were fitted on mixture biomass as a function of species relative proportion. To assess the potential benefits of mixtures, their performance was then compared to the performance expected when species did not interact. In this case, the expected performance is the simple addition of the expected individual performance of each species, computed as the product of their performance as a sole crop and their respective sowing density in the mixture. An ‘overyielding’ effect is observed when the performance of the mixture is higher than that expected. ‘Transgressive overyielding’ appears when the mixture performance exceeds that of the best performing sole crop. To assess the importance of these effects, the frequency of overyielding (fO) was calculated as the percentage of overyielding cases predicted by the polynomial regression for the whole range of species proportion combination (Fig. 1). To further quantify the overyielding effect, overyielding intensity (iO), i.e. the biomass gain due to overyielding on the whole range of proportion combinations, was computed as the ‘surface’ between the polynomial regression and the expected performance (Fig. 1), similarly to what is done to compute signal intensity in chromatograms. Transgressive overyielding frequency (fTO) and intensity (iTO) were calculated in a similar way, taking the biomass of the best performing sole crop as reference. In addition, maximum biomass surplus due to transgressive overyielding (sTO) was computed as the difference between the highest predicted mixture performance and the highest sole crop performance (Fig. 1).

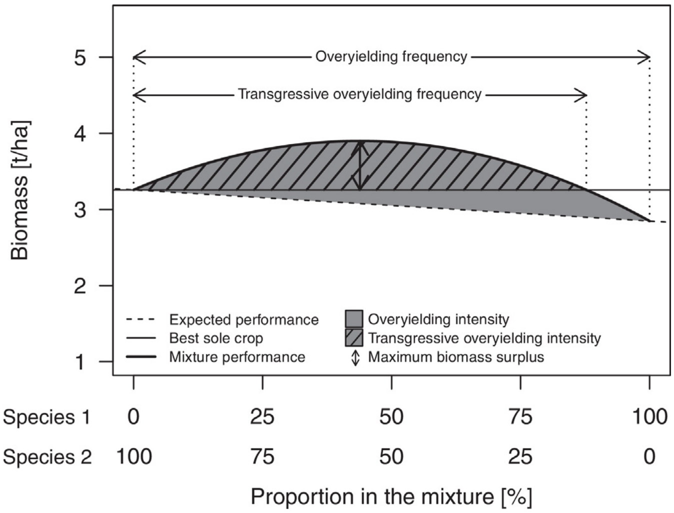

Fig. 1. Explanatory scheme for the determination of overyielding and transgressive overyielding frequency and intensity, and the biomass surplus, i.e. difference between the highest predicted mixture biomass and highest sole crop biomass.

This global approach was compared to the land equivalent ratio (LER) method, an approach that quantifies the biomass benefit or loss of mixtures over sole crops independently for each single mixture observation. The LER measures the relative land area required for a sole crop to produce the same biomass as the mixture (Willey and Osiru, 1972). The LER of the mixture is calculated by adding the partial LER (pLER) of the two species i and j:

$$\text{LER} = \text{pLER}_i + \text{pLER}_j = \frac{Y_{\text{M}i}}{Y_{\text{SC}i}} + \frac{Y_{\text{M}j}}{Y_{\text{SC}j}} \tag{1}$$

where $Y_{\text{M}i}$ is the yield of species i in mixture and $Y_{\text{SC}i}$ is the yield of species i as a sole crop at standard sowing density. An LER above one indicates that the mixture performance is higher than the expected performance in the absence of species interactions.

2.3.2. Species interactions

For each mixture, the relation between plant dry weight and sowing density of both species was investigated using the extended hyperbolic yield-density equation from Wright (1981):

$$w_{ij}^{-1} = b_0 + b_1 d_{ij} + b_2 d_{ji} \quad \text{for species } i \tag{2a}$$

and

$$w_{ji}^{-1} = c_0 + c_1 d_{ji} + c_2 d_{ij} \quad \text{for species } j \tag{2b}$$

where w is the dry weight per plant, d is the effective plant density of the species in the mixture in plant/m^2, and b_0, b_1, b_2, c_0, c_1 and c_2 are constants. b_1 and c_1 describe the effect of intraspecific density, while b_2 and c_2 describe the effect of interspecific density. The ratios b_1/b_2 and c_1/c_2 measure the relative competitive ability (RC) of the species (Helenius and Jokinen, 1994).

For each species as a sole crop, the effect of the sowing density on biomass production was assessed by linear regression. The influence of the identity of the associated species on the focal species biomass production was tested with an analysis of covariance (ancova), with sowing density as the quantitative covariable and associated species as the qualitative factors. Results from the ancova global analysis were compared to those using the ‘partial LER’ approach, which compares the pLER value at a specific density to a reference (‘classical reference’, C_{ref}) based on the expected performance in the absence of species interactions:

$$C_{\text{ref}} = \frac{Y_{\text{SC }100\%} \times \text{SD}}{Y_{\text{SC }100\%}} \quad (3)$$

where $Y_{\text{SC }100\%}$ is the performance of the species as a sole crop at standard sowing density and SD is species sowing density in the mixture. The product of $Y_{\text{SC }100\%}$ and SD is the expected performance of the species in the mixture. For example, for a crop in a 50%–50% mixture, the expected performance would correspond to half the performance as a sole crop at 100%, giving a reference value of 0.5. A pLER value greater than this reference is interpreted as a facilitation effect and a lower value as a negative influence of the associated species.

In addition to this classical reference value, a new reference ('intraspecific response reference', IR_{ref}) is proposed to take into account the intraspecific response of the species to a reduction of its density. This reference is computed using the performance of the species as a sole crop at two different sowing densities:

$$\text{IR}_{\text{ref}} = \frac{Y_{\text{SC }X\%}}{Y_{\text{SC }100\%}} \quad (4)$$

where $Y_{\text{SC }X\%}$ and $Y_{\text{SC }100\%}$ are the performance of the species as a sole crop at the same sowing density as in the mixture and at standard sowing density, respectively.

While, for a mixture with a given relative proportion, the classical reference is the same regardless of the associated species, the intraspecific response reference depends on species intraspecific competition. For example, in a 50%–50% mixture, the second reference is calculated by dividing the performance of the species as sole crop at 50% sowing density by that at 100%. When species performance at 50% of the sowing density is half of that at full sowing density, the two references are equal (0.5) (Fig. S2a, Supporting Information). By contrast, if the performance is higher than half of that at full sowing density the intraspecific response reference is higher than the classical one (Fig. S2b and c, Supporting Information). Above intraspecific response reference, a clear facilitation effect of the associated species is evidenced. By contrast, for values lying between the two references, facilitation effects and a decrease of intraspecific competition could occur together.

Intraspecific response reference was calculated for the four species grown at an equivalent relative proportion (1:1) in the six bispecific mixtures with N fertilisation.

2.3.3. Influence of species interactions on mixture performance

For each of the six mixtures, the influence of species interactions on the performance of the mixture was characterised by three different indices.

(a) Resource complementarity was assessed with the niche differentiation index (NDI) calculated with the competition coefficients (Spitters, 1983):

$$\text{NDI} = \frac{b_1}{b_2} \times \frac{c_1}{c_2} \quad (5)$$

(see formula (2a) and (2b) for the meaning of b_1, b_2, c_1 and c_2).

NDI values greater than one correspond to niche differentiation.

- The relative dominance was evaluated by the disequilibrium in species abundance within each mixture (rAb). It was computed by the relative difference between expected and observed biomass:

$$\text{rAb} = \left|\left|\frac{\text{eY}_i}{\text{eY}_i + \text{eY}_j} - \frac{Y_i}{Y_i + Y_j}\right|\right) \quad (6)$$

where eY_i and eY_j are the expected biomass of species i and j and Y_i and Y_j, their measured biomass.

- Relative competitiveness (rRC) was assessed by the ratio of the relative competitive ability (RC) of the most competitive species on the RC of the less competitive species:

$$\text{rRC} = \frac{b_1}{b_2} \div \frac{c_1}{c_2} \text{ or } \frac{c_1}{c_2} \div \frac{b_1}{b_2} \quad (7)$$

(see formula (2a) and (2b) for the meaning of b_1, b_2, c_1 and c_2).

All statistical analyses were performed with R 3.1.1 (R Core Team, 2014).

3. Results

First, we present an overview of sole crop and mixture performance. The difference between the biomass of the mixtures and that expected from sole crops is characterised and quantified. Second, the influence of the associated species on species biomass production is shown. Finally, the effects of complementarity and differences in competitiveness between species on mixture performance are presented.

3.1. Plant emergence and initial growth rate

Plant emergence was good due to the high water availability during 10 days following the sowing (54.2 mm precipitation). Mustard and pea attained 100% of their targeted plant density. Plant emergence was lower for oat and phacelia (about 65%). After plant emergence and up to the end of September, the cumulative rainfall was relatively low (47.4 mm) and N mineralisation may have been limited.

Mustard showed the fastest initial growth (23% soil cover 20 days after sowing), followed by oat with 17%. Phacelia and pea had the lowest growth rate with 12% and 13% soil cover 20 days after sowing, respectively.

3.2. Overall performance

With N fertilisation, the shoot biomass production of the sole crops ranged from 1.9 t/ha to 3.7 t/ha with an average of 2.7 t/ha (Fig. 2). In comparison, the biomass production of the mixtures was on average higher (3.6 t/ha), ranging from 2.2 t/ha to 5.6 t/ha. Similarly, sole crops accumulated on average 55.1 kg/ha of N (ranging from 30.4 kg/ha to 77.0 kg/ha), while the average of the mixtures was 68.2 kg/ha of N (varying from 34.1 kg/ha to 121.6 kg/ha).

The performance of sole crops and mixtures was generally lower without N fertilisation than with N fertilisation (Table 1). Without N fertilisation, the sole crops produced 2.1 t/ha of biomass (ranging from 1.3 t/ha to 3.0 t/ha) and accumulated 43.1 kg/ha of N (ranging from 25.6 kg/ha to 97.4 kg/ha, Fig. 2). The mixtures produced on average 2.8 t/ha biomass (ranging from 1.6 t/ha to 4.6 t/ha) and accumulated 57.3 kg/ha of N (varying from 27.0 kg/ha to 120.6 kg/ha). Mustard-pea was the only mixture producing a similar biomass with and without N fertilisation and it even accumulated more N without N fertilisation. The difference in N accumulation between the two fertilisation levels was highest for the oat-phacelia mixture, with a mean of 23.3 kg/ha more N with N

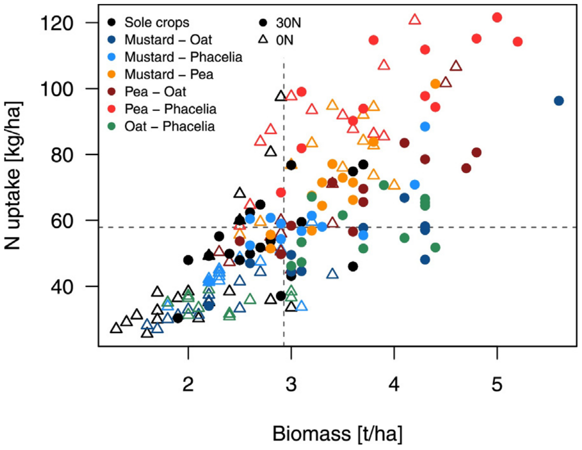

Fig. 2. N uptake in function of shoot biomass production for the sole crops and the mixtures, with and without N fertilisation. The dotted lines represent the average values of all crops ($n = 176$).

fertilisation (average of the values obtained for the three species relative proportions), followed by mustard-oat mixture, with a mean of 19.5 kg/ha greater N accumulation (Table 1). However, these differences in accumulated N did not reach the amount of supplied N (30 kg N/ha), except for oat-phacelia with a 1:3 relative proportion.

3.3. Comparison of mixture and sole crop performance

Mixture sowing density had no influence on biomass production ($p > 0.05$) at both N fertilisation levels, so the polynomial models were adjusted with all mixture sowing densities confounded.

With N fertilisation, the best fitting model was a 2nd or 3rd degree polynomial model for all mixtures, except mustard-phacelia (i.e. for five mixtures out of six) (Fig. 3). These five mixtures showed 'overyielding' for most of the range of species proportion combinations (overyielding frequency fO = 79% for mustard-oat and fO = 100% for the other four mixtures), while mustard-phacelia exhibited none (Table S1, Supporting Information). The five mixtures even exhibited 'transgressive overyielding', meaning that they produced more biomass than the best performing sole crop, in more than 70% of the range (fTO > 70%). The extent of transgressive overyielding varied according to mixtures. Pea-oat had the highest transgressive overyielding intensity (iTO = 66.5), while mustard-oat had the highest biomass surplus (sTO = 1.36 t/ha). By contrast, oat-phacelia showed the lowest transgressive overyielding intensity (iTO = 37.6) and the lowest biomass surplus (sTO = 0.64 t/ha). For mustard-phacelia, all the terms of the polynomial regression were not significantly different from 0. This means that in average, the two sole crops and the mixtures produced the same amount of biomass (overall mean of 3.1 t/ha, Fig. 3b).

The interpretation of LER values resulted in similar insights with respect to overyielding. With N fertilisation, all the mixtures, except one, presented a LER higher than 1, meaning that the mixtures superseded the expected performance (Table 2). Among

Table 1
Total and specific biomass and N uptake of the cover crops for the two N fertilisation levels, and differences between the two fertilisation levels (mean value of the four mixture sowing densities). Species 1 is the first species mentioned in the species proportion and species 2 is the second one.

	Species proportion	Biomass [t/ha]							N uptake [kg/ha]						
		30N			0N			Mean difference (30N – 0N)	30N			0N			Mean difference (30N – 0N)
		Species 1	Species 2	Mixture	Species 1	Species 2	Mixture		Species 1	Species 2	Mixture	Species 1	Species 2	Mixture	
Mustard	1	2.58			2.05			0.53	50.4			36.9			13.5
Pea	1	2.70			2.68			0.03	66.0			76.5			−10.5
Oat	1	2.85			2.38			0.48	39.6			31.3			8.3
Phacelia	1	2.90			1.70			1.20	58.0			31.1			26.9
Mustard–oat	1:3	2.35	1.98	4.33	0.75	1.55	2.30	2.03	37.6	24.2	61.8	12.8	23.4	36.3	25.6
	1:1	2.40	1.20	3.60	1.00	1.15	2.15	1.45	39.3	15.6	54.9	16.3	16.2	32.5	22.4
	3:1	2.05	0.83	2.88	1.28	0.93	2.20	0.68	34.4	11.4	45.8	21.3	14.0	35.3	10.5
Mustard–phacelia	1:3	1.95	1.60	3.55	1.60	1.05	2.65	0.90	33.1	28.1	61.2	30.0	19.2	49.2	11.9
	1:1	1.98	0.68	2.65	1.75	0.63	2.38	0.28	41.2	12.7	53.8	32.8	11.5	44.2	9.6
	3:1	2.70	0.55	3.25	2.00	0.43	2.43	0.83	54.8	10.2	65.0	31.4	8.1	39.5	25.4
Mustard–pea	1:3	2.55	1.05	3.60	1.38	2.18	3.55	0.05	52.8	26.7	79.5	25.7	61.2	86.8	−7.3
	1:1	2.60	0.73	3.33	1.50	1.78	3.28	0.05	47.9	18.6	66.5	26.9	50.9	77.8	−11.3
	3:1	2.70	0.60	3.30	2.25	1.20	3.45	−0.15	50.8	13.4	64.2	36.8	34.4	71.2	−7.0
Pea–oat	1:3	0.83	2.68	3.50	0.85	2.15	3.00	0.50	20.0	41.1	61.1	20.5	32.3	52.8	8.3
	1:1	1.25	2.68	3.93	1.10	1.80	2.90	1.03	30.6	39.0	69.6	27.5	28.7	56.2	13.4
	3:1	1.15	2.38	3.53	1.88	1.70	3.58	−0.05	28.5	40.1	68.6	49.3	30.3	79.6	−11.0
Pea–phacelia	1:3	1.20	3.30	4.50	1.85	1.75	3.60	0.90	33.0	70.5	103.5	58.5	37.8	96.3	7.2
	1:1	1.78	2.43	4.20	1.90	1.18	3.08	1.13	53.1	53.9	107.0	55.2	26.8	82.0	25.0
	3:1	1.68	1.68	3.35	2.20	1.05	3.25	0.10	48.9	41.4	90.3	64.9	22.7	87.6	2.7
Oat–phacelia	1:3	1.38	2.35	3.73	1.00	0.95	1.95	1.78	17.9	48.3	66.2	16.2	20.3	36.6	29.7
	1:1	2.28	1.68	3.95	1.78	0.65	2.43	1.53	26.1	33.7	59.8	21.8	13.8	35.6	24.2
	3:1	2.70	0.85	3.55	1.93	0.53	2.45	1.10	30.9	18.4	49.2	22.8	10.2	33.1	16.2

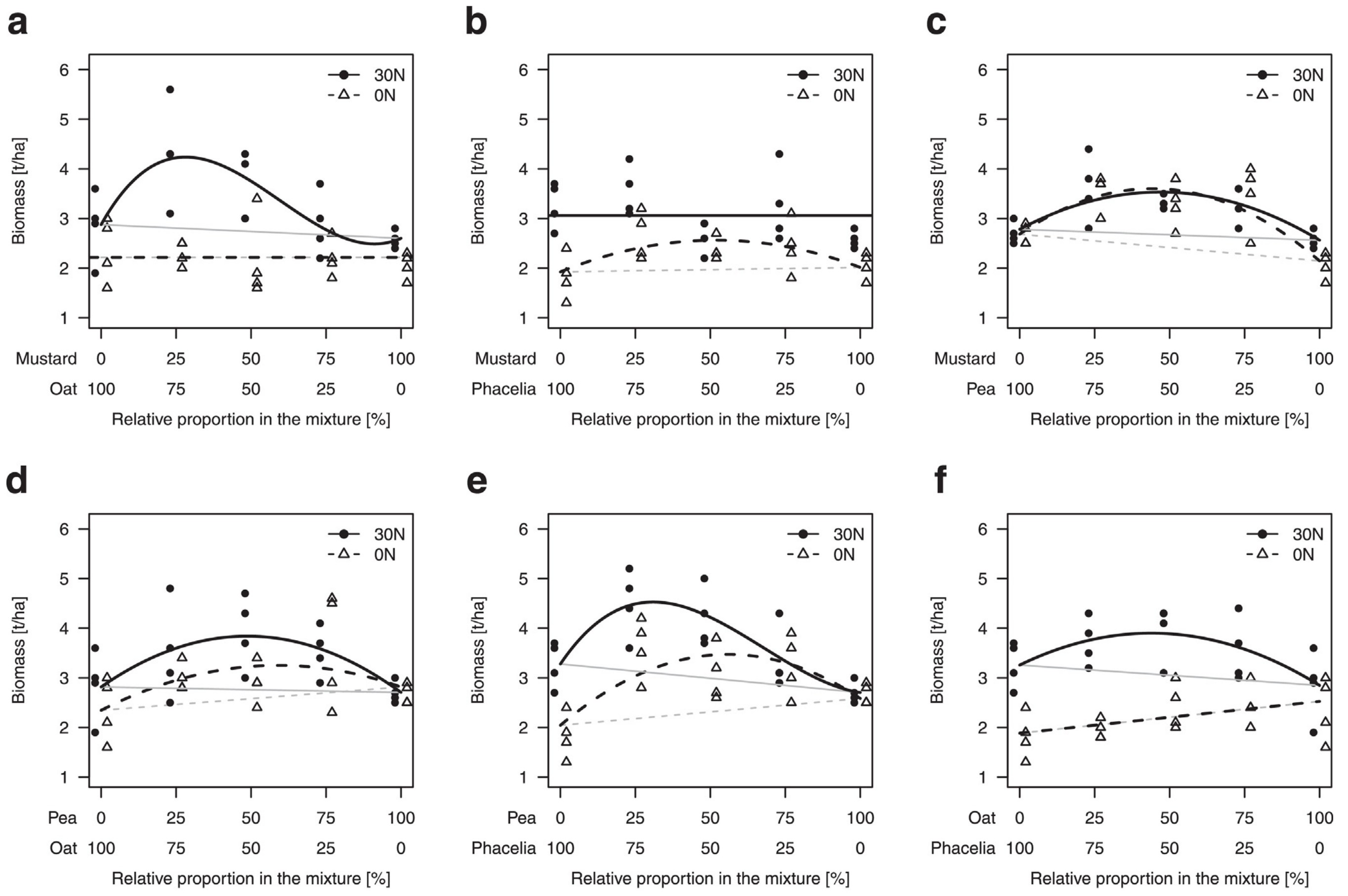

Fig. 3. Shoot biomass in function of species relative proportion with and without N fertilisation, for the six mixtures: (a) mustard–oat, (b) mustard–phacelia, (c) mustard–pea, (d) pea–oat, (e) pea–phacelia and (f) oat–phacelia. The black lines represent the polynomial models adjusted on the biomass for the four total mixture density together. The grey lines indicate the expected mixture biomass when species do not interact (see Section 2).

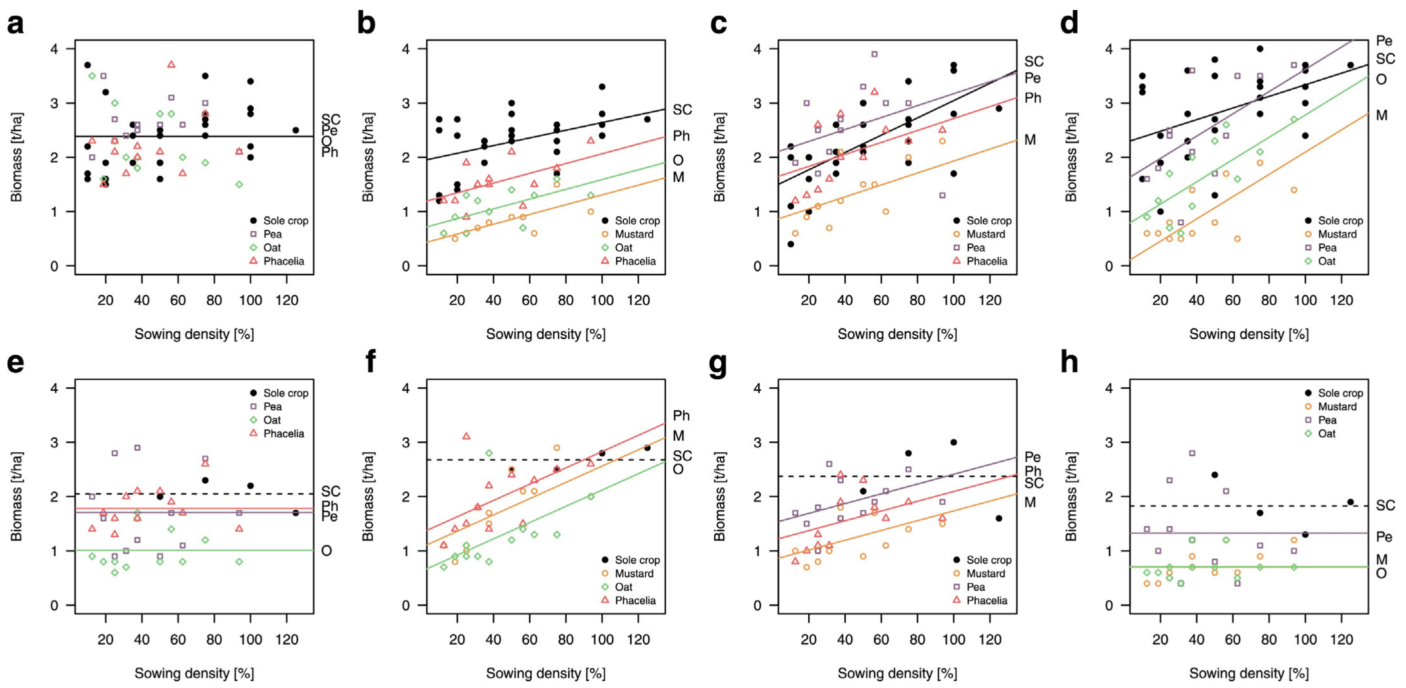

Fig. 4. Shoot biomass in function of the sowing density and of the associated species for (a) mustard, (b) pea, (c) oat and (d) phacelia with N fertilisation, and for (e) mustard, (f) pea, (g) oat and (h) phacelia without N fertilisation. With N fertilisation, the values from the complementary experiment (8 sowing densities and 4 replicates) were included. The lines represent the results from the linear regressions and ancova analysis (see Table 3). SC: sole crop; M: mustard; Pe: pea; O: oat; Ph: phacelia.

Table 2
Partial LER and LER (mean and standard deviation computed on the four mixture sowing densities) for the six mixtures with and without N fertilisation.

Mixture	Species proportion	30N					0N				
		Mustard	Oat	Phacelia	Pea	Mixture	Mustard	Oat	Phacelia	Pea	Mixture
Mustard–oat	1:3[a]	0.92 ± 0.37	0.71 ± 0.11			1.63 ± 0.36[c]	0.37 ± 0.08	0.69 ± 0.20			1.06 ± 0.27
	1:1[b]	0.94 ± 0.25	0.44 ± 0.13			1.38 ± 0.20	0.48 ± 0.15	0.50 ± 0.19			0.99 ± 0.32
	3:1[a]	0.80 ± 0.21	0.31 ± 0.12			1.10 ± 0.31	0.62 ± 0.16	0.42 ± 0.16			1.04 ± 0.22
Mustard–phacelia	1:3	0.76 ± 0.17		0.49 ± 0.08		1.25 ± 0.19	0.80 ± 0.26		0.66 ± 0.23		1.46 ± 0.34
	1:1	0.77 ± 0.08		0.21 ± 0.07		0.98 ± 0.15	0.86 ± 0.14		0.39 ± 0.13		1.25 ± 0.16
	3:1	1.04 ± 0.26		0.17 ± 0.04		1.22 ± 0.28	0.97 ± 0.18		0.27 ± 0.10		1.24 ± 0.23
Mustard–pea	1:3	0.99 ± 0.25			0.39 ± 0.13	1.38 ± 0.25	0.67 ± 0.25			0.81 ± 0.17	1.48 ± 0.17
	1:1	1.01 ± 0.09			0.27 ± 0.07	1.29 ± 0.03	0.74 ± 0.45			0.65 ± 0.21	1.4 ± 0.29
	3:1	1.05 ± 0.15			0.22 ± 0.03	1.27 ± 0.12	1.10 ± 0.30			0.44 ± 0.13	1.55 ± 0.35
Pea–oat	1:3		1.04 ± 0.7		0.31 ± 0.11	1.35 ± 0.71		0.95 ± 0.23		0.32 ± 0.03	1.27 ± 0.21
	1:1		0.98 ± 0.35		0.46 ± 0.06	1.45 ± 0.34		0.83 ± 0.35		0.41 ± 0.06	1.24 ± 0.39
	3:1		0.91 ± 0.45		0.43 ± 0.15	1.34 ± 0.38		0.83 ± 0.57		0.71 ± 0.29	1.53 ± 0.71
Pea–phacelia	1:3			1.02 ± 0.23	0.45 ± 0.09	1.47 ± 0.22			1.04 ± 0.46	0.68 ± 0.29	1.72 ± 0.45
	1:1			0.75 ± 0.22	0.66 ± 0.11	1.41 ± 0.12			0.68 ± 0.35	0.70 ± 0.14	1.38 ± 0.25
	3:1			0.52 ± 0.21	0.62 ± 0.19	1.14 ± 0.17			0.66 ± 0.36	0.82 ± 0.15	1.48 ± 0.36
Oat–phacelia	1:3		0.51 ± 0.14	0.72 ± 0.11		1.23 ± 0.23		0.45 ± 0.16	0.58 ± 0.24		1.03 ± 0.18
	1:1		0.83 ± 0.21	0.51 ± 0.14		1.35 ± 0.13		0.77 ± 0.17	0.41 ± 0.15		1.17 ± 0.18
	3:1		1.03 ± 0.45	0.27 ± 0.11		1.30 ± 0.54		0.85 ± 0.26	0.33 ± 0.12		1.18 ± 0.16

[a] For 1:3 mixtures, partial LER classical reference for interpretation is 0.25 for species 1 and 0.75 for species 2 and conversely for 3:1 mixtures.
[b] For 1:1 mixtures, partial LER classical reference is 0.5 for both species.
[c] Classical reference for LER interpretation is 1.

the six bispecific mixture types, mustard-phacelia showed the lowest LER values. The LER of mustard-phacelia were close to 1 or higher, but lower than for the other mixtures.

With N fertilisation, mixture biomass was significantly influenced by species relative proportion only for mustard-oat and pea-phacelia ($p = 0.02$ and $p = 0.03$, respectively). In the mustard-oat mixture, a high proportion of oat produced significantly more biomass than a high proportion of mustard. For the pea-phacelia mixture, the biomass production was significantly greater with a high proportion of phacelia compared to a 1:1 proportion or compared to a high proportion of pea.

Without N fertilisation, the quadratic model provided the best fit for most mixtures. For four mixtures out of six, i.e. for the three mixtures including pea and for the mustard-phacelia mixture, overyielding was observed for the whole range of species proportion combinations (Fig. 3 and Table S1, Supporting Information). In addition, for these mixtures, the transgressive overyielding frequency fTO was greater than 80%. Mustard-pea showed the highest transgressive overyielding intensity (iTO = 54.1) and the highest biomass surplus (sTO = 0.92 t/ha). Pea-oat had the lowest values, with iTO = 23.8 and sTO = 0.44 t/ha, respectively. For oat-phacelia, the best fitting model was a linear model (Fig. 3f). The predicted mixture biomass was thus lower than that of the best performing sole crop (oat: 2.5 t/ha). In mustard-oat, none of the terms of the quadratic model were significantly different from 0. Biomass production was thus similar in sole crops and in mixture whatever the relative proportion of both species (2.2 t/ha, Fig. 3a).

Without N fertilisation, all LER values were also higher than 1 (Table 2). Mustard-oat and oat-phacelia showed the lowest LER (respectively, 1.03 and 1.13 on average), while pea-phacelia had the highest (1.53 on average).

Table 3
Linear regression (sole crops) of biomass in function of sowing density and analysis of covariance (mixtures) of biomass in function of sowing density ('Density', covariable) and the identity of the associated species ('Associated species', qualitative factor). Models were simplified to keep only the significant terms.

	N [kg/ha]		Mustard			Pea			Oat			Phacelia		
			df	MS	*p*	*df*	MS	*p*	*df*	MS	*p*	*df*	MS	*p*
Sole crops	30	Density	–	–		1	1.59	0.011	1	8.05	<0.001	1	3.63	0.015
		Error	26	0.38		26	5.45		26	0.35		26	0.53	
	0	Density	–	–		–	–		–	–		–	–	
		Error	3	0.07		3	0.04		3	0.42		3	0.21	
Mixtures	30	Density	–	–		1	1.52	<0.001	1	2.60	0.010	1	8.28	<0.001
		Associated species	–	–		2	1.76	<0.001	2	4.73	<0.001	2	7.00	<0.001
		Error	35	0.32		32	0.08		32	0.35		32	0.32	
	0	Density	–	–		1	4.40	<0.001	1	1.43	0.006	–	–	
		Associated species	2	4.39	0.002	2	1.54	0.006	2	1.37	0.001	2	1.54	0.004
		Error	33	9.18		32	0.26		32	0.16		33	0.23	

df, degree of freedom; MS, mean square; *p*, *p* value.

3.4. Species effect on the performance of the associated species and competition coefficients

The four species showed contrasting responses to increasing sowing density (Fig. 4). With N fertilisation, the biomass of mustard as a sole crop was not affected by the sowing density ($p = 0.085$). By contrast, the yield of pea, oat and phacelia was highly density dependent ($p < 0.05$). We observed the same positive effect of the sowing density in sole crops and in mixtures. The slope of the linear regressions of biomass on sowing density determined with the ancova analysis was 0.009 for pea ($p = 0.011$), 0.011 for oat ($p < 0.001$) and 0.022 for phacelia ($p = 0.015$).

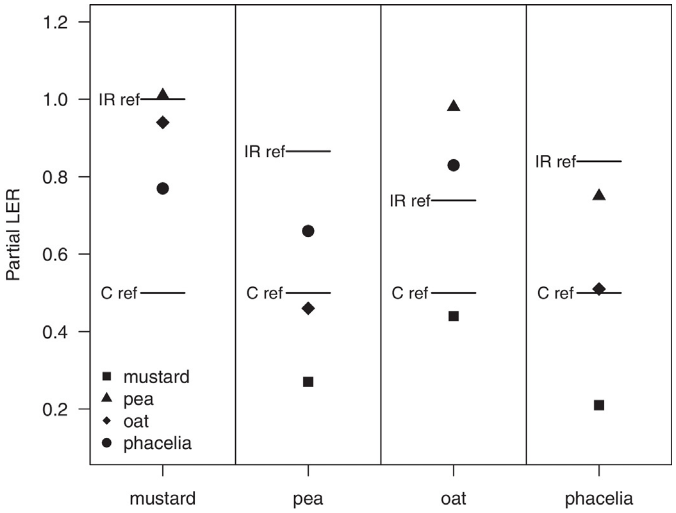

Fig. 5. Partial LER, classical reference (C ref) and the intraspecific response reference (IR ref) computed for the four species in bispecific mixtures at equivalent proportion with each of the three other species, in the case with N fertilisation.

Mustard produced the same biomass, with an average of 2.4 t/ha, regardless of the associated species ($p = 0.166$; Fig. 4a). The biomass production of the three other species depended on the associated species ($p < 0.001$, Fig. 4b–d and Table 3). A stimulating effect of pea on phacelia and oat was observed, i.e. species produced as much (phacelia) or even more (oat) biomass in combination with pea compared to their production as sole crops. By contrast, mustard strongly limited the performance of the focal species and to a greater extent than the other associated species. The biomass production of phacelia was 85% lower with mustard than with pea. The effect of oat and phacelia on the focal species was less pronounced compared to that of pea and mustard, but phacelia was less limiting than oat.

Partial LER values showed the same tendencies but were highly variable (Table 2). The pLER for mustard were always higher than the values expected in absence of interspecific competition (classical reference). For the other species, pLER were higher than the classical reference when they were associated with pea or phacelia. By contrast, when associated with mustard, pLER were lower (phacelia) or close to the classical reference value (oat). With oat, pLER for pea and for phacelia were generally close to that expected.

When comparing the results to two references (classical and intraspecific response reference), pLER of the species associated with pea were always higher than this reference, while species in mixture with phacelia and oat generally showed pLER between the two references (Fig. 5).

Without N fertilisation, the sowing density had no effect on the performance of mustard ($p = 0.513$) and phacelia ($p = 0.690$). On the contrary, the identity of the associated species significantly influenced the performance of all species ($p < 0.05$, Table 3). Similarly to fertilised conditions, pea had a positive influence on

Table 4
Parameter values and coefficient of determination of the hyperbolic yield-density equation of the six mixtures with and without N fertilisation. b_1 and c_1 correspond to the intraspecific competition coefficients of species 1 and 2, respectively, and b_2 and c_2 are the interspecific competition coefficients. RC represents the species relative competitive ability, rRC the relative competitiveness and NDI the niche differentiation index.

N [kg/ha]	Mixture	Species	b_0 c_0	b_1 c_1	b_2 c_2	r^2	RC	rRC	NDI
30	Mustard–oat	Mustard	−0.277	0.00494***	0.00192	0.84	2.58	1.2	5.4
		Oat	0.090	0.00342***	0.00162**	0.61	2.11		
	Mustard–phacelia	Mustard	−0.158	0.00424***	0.00170*	0.91	2.49	2.9	2.2
		Phacelia	−0.254	0.00463*	0.00534**	0.55	0.87		
	Mustard–pea	Mustard	−0.100	0.00410***	0.00086	0.96	4.76	1.5	14.7
		Pea	0.077	0.00483*	0.00157*	0.40	3.08		
	Pea–oat	Pea	0.201	0.00360*	0.00098	0.30	3.67	1.2	16.1
		Oat	−0.099	0.00442**	0.00101	0.62	4.39		
	Pea–phacelia	Pea	0.091	0.00358***	0.00050	0.64	7.10	9.1	5.5
		Phacelia	0.042	0.00257**	0.00331	0.55	0.78		
	Oat–phacelia	Oat	0.059	0.00323***	0.00059	0.82	5.49	8.1	3.7
		Phacelia	0.023	0.00279***	0.00411***	0.71	0.68		
0	Mustard–oat	Mustard	−1.005	0.00852***	0.01155**	0.72	0.74	4.9	2.7
		Oat	−0.110	0.00575***	0.00157*	0.71	3.65		
	Mustard–phacelia	Mustard	−0.256	0.00583***	0.00138	0.88	4.22	2.8	6.3
		Phacelia	0.003	0.00664***	0.00444***	0.73	1.50		
	Mustard–pea	Mustard	−0.682	0.00654***	0.01148**	0.82	0.57	44.1	14.3
		Pea	0.169*	0.00259***	0.00010	0.74	25.10		
	Pea–oat	Pea	0.092	0.00358**	0.00151*	0.56	2.38	1.5	8.6
		Oat	−0.077	0.00509***	0.00141	0.80	3.62		
	Pea–phacelia	Pea	0.154*	0.00277***	0.00014	0.82	20.42	37.8	11.0
		Phacelia	−1.558*	0.01181***	0.02188**	0.67	0.54		
	Oat–phacelia	Oat	−0.134	0.00532***	0.00140	0.81	3.81	3.9	3.7
		Phacelia	−0.852	0.01059***	0.01083**	0.70	0.98		

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

the focal species performance (Fig. 4e, g and h). Without N fertilisation, oat was the most limiting species. The biomasses of mustard and pea were 46% and 66% lower in mixture with oat than in mixture with phacelia, respectively. Mustard and oat had comparable effects on phacelia (insignificant difference between intercepts, $p = 0.966$). By contrast, mustard had no effect on pea. Similar results were observed with pLER, species had higher pLER than the classical reference in mixture with pea, while with oat, pLER were lower than expected at low relative proportion of oat (Table 2).

Using the relative competitive ability of the species (RC, Table 4), the following hierarchy of the four species was established for the N fertilised conditions: mustard > oat > pea > phacelia, with mustard being the most competitive species and phacelia the least. The interspecific competition coefficients (b_2 and c_2) were generally lower than the intraspecific competition coefficients (b_1 and c_1), except for phacelia. The competitive ability of mustard was lower without N than with N fertilisation, whereas that of oat was higher without N fertilisation. The competitive hierarchy of the four species was therefore different without N fertilisation: oat > pea > mustard > phacelia (see RC Table 4).

3.5. Influence of species interactions on mixture performance

All the mixtures showed a niche differentiation index (NDI) greater than 1 indicating that the species exhibited resource use complementarity (Table 4). Without N fertilisation, a high positive correlation was observed between mixture biomass and NDI (Kendall's coefficient of correlation $\tau = 1$, $p < 0.001$, Fig. 6a). The highest performance was achieved by the mixture exhibiting the highest resource complementarity (mustard-pea). By contrast, with N fertilisation, no relationship was observed ($\tau = 0.2$, $p = 0.719$).

For five out of the six mixtures, the relative abundance of the dominant species was higher in fertilised conditions than in unfertilised conditions (Fig. 6b). With N fertilisation, a negative correlation was found between mixture biomass and the relative dominance of species in the mixture ($\tau = -0.73$, $p = 0.06$). The highest mixture performances were thus related to low disequilibrium in species abundance. Without N fertilisation, no relation was found ($\tau = -0.07$, $p = 1$).

The relationship between relative competitiveness (rRC) and mixture performance was less clear (Fig. 6c). For low rRC (<5), a negative correlation between performance and rRC was observed. However, four mixtures with a rRC > 5 (mustard-pea without N, oat-phacelia with N and pea-phacelia for both N fertilisation levels) reached a high performance. Each of these four mixtures contained one species with a very low interspecific competition coefficient (b_2 or c_2, Table 4).

4. Discussion

4.1. Interspecific interactions

Contrasting effects of the associated species were observed among the four cover crop species grown in bispecific mixtures. Facilitation effects were observed when the species were associated with pea and phacelia. By contrast, mustard in fertilised and oat in unfertilised conditions had a negative effect on the associated species.

In mixture with pea, species produced as much biomass as in sole crop, or even more, despite the presence of pea. As legume species can access atmospheric N through biological fixation, the associated species had likely access to higher amounts of soil N in association with pea than with non-legume species.

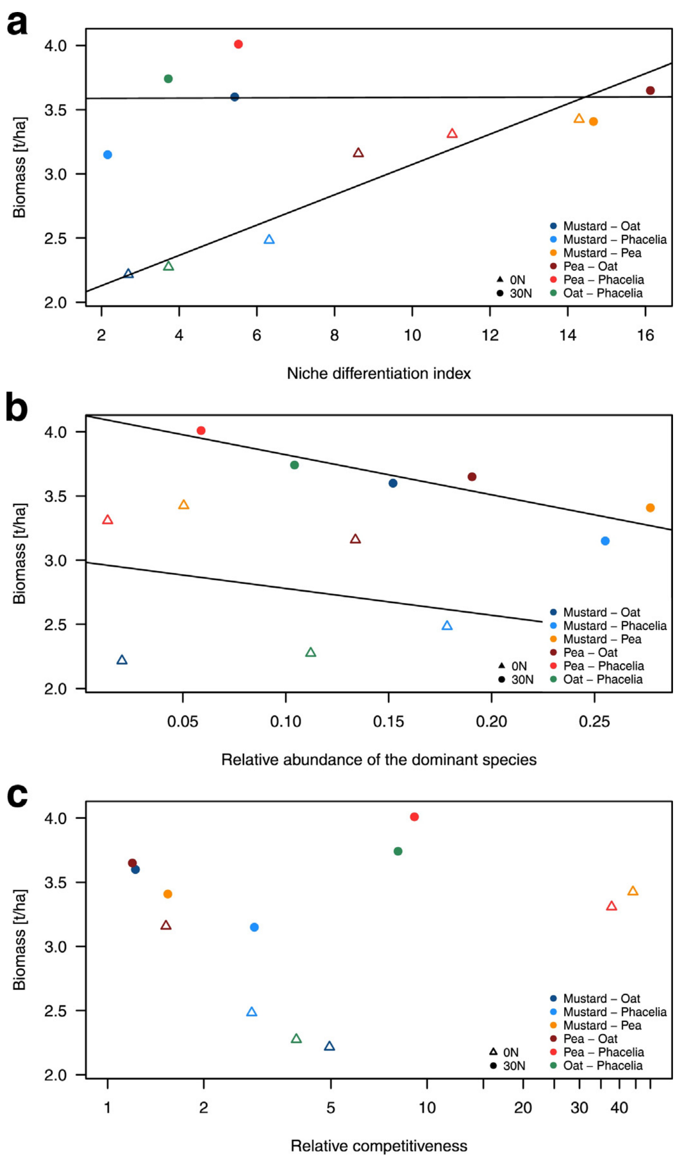

Fig. 6. Shoot biomass of the six mixtures with and without N fertilisation in function of (a) niche differentiation index (NDI), (b) species relative abundance and (c) relative competitiveness (rRC). The relative competitiveness is given on a logarithmic scale.

The effect of the non-legume species on the other species was strongly dependent on their competitive ability. Species with low competitive ability, such as phacelia, had a positive or a neutral influence on more competitive associated species, which produced as much biomass as in sole crop. More competitive species had generally high intraspecific competition coefficient, meaning that their performance as sole crops could be limited despite increasing sowing density, as it was observed here for mustard. Replacing part of a competitive species by another, less competitive, should allow to reduce competition in mixture. As a result, the more competitive species should be able to produce as much biomass in mixture as in sole crop, or to increase its performance despite the presence of another species requiring the same soil resources. This was observed by Wortman et al. (2012) for mustard in mixture with other species. They suggested that mustard creates a very competitive environment for light resources and that replacing part of the highly light competitive mustard by another less competitive species leads to a higher biomass production of mustard.

The most competitive species had a negative influence on the other species, leading to a large decrease in its biomass production. Without N fertilisation, oat was the most competitive species. One explanation could be its higher root length density, especially in deeper soil layers (Wendling et al., 2016). With higher root lengths, oat may explore more soil and thus access greater amounts of nutrients, particularly N, which was probably limiting in unfertilised conditions.

In fertilised conditions, mustard was the most competitive species and was highly dominant in the mixtures. N fertilisation is known to modify species competitiveness (e.g. Andersen et al., 2004; Corre-Hellou et al., 2006; Hauggaard-Nielsen and Jensen, 2001), mainly because the supply of a limiting resource, such as N, favours the growth of the dominant species, and thereby affects negatively the growth of less competitive species. The competitive advantage of mustard over the other species could be due to the very quick establishment of this species. Indeed, mustard showed higher soil cover percentage 20 days after sowing than all the other studied species, even higher than pea which was expected to better cover the soil as it is a creeping plant. This very fast initial growth rate may have conferred a competitive advantage to the mustard for resource acquisition. In addition, several studies have highlighted the allelopathic potential of Brassicaceae (e.g. Haramoto and Gallandt, 2005), which could have limited the growth of the other species.

4.2. Performance of the mixtures

One objective of this study was to evaluate the performance of mixtures compared to sole crops. Several services provided by cover crops can be increased by maximising their biomass production (Finney et al., 2016). Mirsky et al. (2013) observed, for example, that weed suppression was highly affected by the biomass of the cover crop. However, several studies showed that an increase in biomass production did not lead to increased ecosystem services (Smith et al., 2014; Wortman et al., 2012). In these studies, the mixture did not produce more biomass than the most productive sole crop. For this reason, transgressive overyielding, where mixture performance exceeds that of the highest performing sole crop, should be more interesting than just overyielding (i.e. mixture performance higher than the mean performance of sole crops). Indeed, Finney et al. (2016) suggested that biomass-dependent services can only be higher in mixture than in sole crop if the mixture exhibit transgressive overyielding. Observations of transgressive overyielding in cover crop mixtures are limited (e.g. Alonso-Ayuso et al., 2014; Hayden et al., 2014). Nevertheless, in this experiment, we observed that most of the mixtures exhibited transgressive overyielding. Mixture performance was generally higher than that of sole crops, both in terms of biomass and N uptake.

Several factors affecting the performance of the mixture were evidenced in this study. Complementarity is a key mechanism for increasing biomass production and can be assessed using the niche differentiation index (NDI, Spitters, 1983). Without N fertilisation, a high positive correlation was found between mixture biomass production and NDI. By contrast, no relationship was observed with N fertilisation. This highlights that resource complementarity is particularly important when resources are limited. In fact, without N, the mixtures producing the highest biomass were those showing the highest NDI values. The most productive mixtures were the mixtures containing pea. Complementary resource use has been largely reported for mixtures associating legume and non-legume species. Jensen (1996) and Hauggaard-Nielsen et al. (2001a) showed a more efficient resource use in this type of mixtures. In mixture, legume species need to rely more on symbiotic N fixation, since non-legume species are often strong competitor for soil N, mainly due to higher root length density (Li et al., 2006). In addition, some species such as maize could promote N fixation by the associated legume species (Li et al., 2016). Several legume species used as cover crops are able to fix large amounts of atmospheric N in a short period of time (Büchi et al., 2015). Mixtures of legume and non-legume species are thus particularly adapted to low N-input systems (Bedoussac and Justes, 2010). In our experiment, complementarity for N sources allowed the mustard-pea mixture to produce the same biomass without N fertilisation as in fertilised conditions and to accumulate even more N. Complementarity was also observed for all mixtures combining two non-legume species, but to a lower extent. Indeed, complementarity in resource use can also occur along the soil profile, between species with different rooting depths (Hauggaard-Nielsen et al., 2001b). Complementarity can also concern other resources than N, such as phosphorus (Li et al., 2008).

The relative abundance of each species in mixture was strongly unbalanced with N fertilisation. This can lead to a partial loss of the benefits related to complementarity in the mixture as the dominant species tends to suppress the associated species (Andersen et al., 2004; Corre-Hellou et al., 2006; Hauggaard-Nielsen and Jensen, 2001; Neumann et al., 2009; Schmidtke et al., 2004). Indeed, with N fertilisation, we observed a negative correlation between mixture performance and the relative dominance of one species in the mixture, while no relationship was found without N fertilisation. In order to avoid this situation in fertile conditions, mixtures should combine species that have similar competitive abilities. Overall, in our study, the good performance of a mixture was associated with a low competitive ratio except for four mixtures (containing pea or a species with a very low relative competitive ability).

4.3. Methods to assess mixture performance and interspecific interactions

The LER is frequently used to assess mixture advantage over sole crops (Bedoussac and Justes, 2011; Yu et al., 2015). Values greater than one indicate that the mixture produced more biomass than the weighted mean of the sole crops but do not allow detecting whether the mixture is more productive than the best-performing species. As suggested by Garnier et al. (1997), an alternative for mixture assessment using the LER approach would be to calculate the LER of the mixture with respect to the performance of the most productive species. Using this method of LER calculation, an LER greater than one would indicate a transgressive overyielding.

Partial LER (pLER) has been widely used to investigate the effect of species on the performance of the associated species in replacement series (e.g. Bedoussac and Justes, 2010). Values higher than the reference (‘classical reference’) are generally interpreted as facilitation effect, and lower values as a negative influence of the associated species. This approach is based on the assumption that biomass production increases proportionally with the sowing density. However, in our study, we observed that biomass can be constant regardless of the sowing density. Moreover, the comparison to the classical reference value only allows investigating the net effect of competition without dissociating the effects of intra- and interspecific competition. Indeed, a better performance of species in mixture compared to the expectation could be due either to a decrease in intraspecific competition, or to a beneficial effect of the associated species, or both. For this reason, we propose to compare the pLER to two references instead of one, allowing to better separate these two effects. In addition to the classical reference value, an ‘intraspecific response reference’ based on species performance as a sole crop was computed. While for pea and mustard, the conclusions based on the two references were similar to that using only the classical reference (facilitation and negative influence, respectively) the interpretation was less clear for the influence of phacelia on pea or mustard. For these species, facilitation effect of phacelia occurred likely together with a decrease of intraspecific competition. These cases show that it is crucial to test several sowing densities of sole crops to obtain deeper insights on these complex interactions. The use of the method based on two references needs only little additional investment (at least growing the two sole crops at their sowing density in the mixture) and allows concluding more accurately on species interactions.

5. Conclusions

Contrasting interactions between the four cover crop species were evidenced. Facilitation effects were observed in mixtures containing pea: species produced as much biomass as in sole crop or even more. Positive influence was also observed on species associated with species having low competitive ability. By contrast, the most competitive species, oat without N fertilisation and mustard with N fertilisation, strongly limited the biomass production of the associated species.

The characterisation and the quantification of species interactions allowed identifying the key mechanisms affecting mixture performance. Without N fertilisation, complementary resource use had a major role in mixture biomass production. The highest performance was observed in the mixtures containing pea, which showed the highest complementarity. By contrast, with N fertilisation, resource partitioning was less important and mixture biomass production was more affected by the relative dominance of the species in the mixture. Performance was higher when one species was not too dominant relatively to the other, as a highly dominant species suppresses the associated species. Overall, the difference in competitive ability of the species should not be too important to ensure a good performance of the mixture.

This study evidenced that mixtures can produce more biomass than the most-performing sole crop and thus exhibit transgressive overyielding regardless of the N availability. As most of the services provided by cover crops are driven by biomass production, mixtures should thus be considered as a strategy to increase these services.

A new approach to use the LER in the context of cover crop mixtures has also been proposed in this study. To assess the advantage of cover crop mixture over sole crops, the LER should be computed using the best sole crop in order to evidence transgressive overyielding cases, as it has been done in diversity experiments. For the characterisation of species influence on the associated species using the partial LER, a new reference has been proposed in order to separate the effects of facilitation from the effects of a decrease in intraspecific competition.

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Appendix A. Supplementary data

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