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Winter oilseed rape intercropped with complex service plant mixtures: Do all species matter?

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A R T I C L E I N F O

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A B S T R A C T

In the last decade, intercropping of frost-sensitive service plants with winter oilseed rape has become common in Western Switzerland and France. Currently, many farmers grow increasingly complex service plant mixtures. However, the composition of these mixtures is often based on empirical experiments, and little is known about the role of each species in these complex intercropping systems. In this study, we addressed this question by performing two experiments in field conditions. The first consisted of removing one service plant species from a four-legume mixture intercropped with oilseed rape. This four-species mixture consisted of berseem clover, grass pea, lentil, and common vetch. In the second experiment, a fifth species was added to the mixture. The added species was either a legume such as faba bean or a non-legume such as niger, buckwheat, or mustard. Our results show that the specific composition, especially the addition of a non-legume, could strongly affect the fall biomass production of oilseed rape and service plants and potential ecosystem service provision. The addition of buckwheat or mustard to a legume service plant mixture increased early ground cover and service plant dry weight which are known to contribute to weed control. However, it also decreased oilseed rape dry weight in late fall by 29 to 66% and did not favour total nitrogen accumulation by the plant cover. In contrast, the addition or removal of a legume service plant had a limited impact on plant growth and ground cover. Finally, our results highlight that some dominant species could have a big impact on the intercropping growth even when included in small proportion in a complex mixture. The impact of specific composition on cover growth also varied depending on the growing conditions. It remained a key factor in explaining the fall biomass acquisition of service plants and oilseed rape.

1. Introduction

Intercropping is a centuries-old practice implemented worldwide that consists in growing two or more crop species together during at least a part of their life cycle (Vandermeer, 1989; Willey, 1979). Intercropping can increase productivity, improve weed control, and enhance resource use efficiency (Bedoussac et al., 2015; Brooker et al., 2015; Corre-Hellou et al., 2011; Li et al., 2014). After the green revolution, this practice was largely abandoned with the development of intensive agriculture. However, as concerns about the environmental impact of intensive agriculture grew, there was a resurgence of interest in intercropping (Altieri et al., 1983; Duru et al., 2015; Martin-Guay et al., 2018).

One way of implementing intercropping and increasing crop diversity is to grow a cash crop with service plants (SPs). SPs are crops grown to provide ecosystem services and are not harvested (Gardarin et al., 2022; Verret et al., 2017b). These SPs can also be found referred to as living mulch, companion plants, subsidiary crops, or cover crops in the literature; they are often legume species (Gardarin et al., 2022; Hiltbrunner et al., 2007; Salonen and Ketoja, 2020; Verret et al., 2017b).

In complex grassland mixtures, Hector et al. (1999) reported that legume species have a stronger influence on biomass production than other functional groups. This was explained by the legume's ability to enhance niche complementarity through N_2 fixation. This positive effect of legumes was also observed in annual bispecific intercropping systems (Corre-Hellou et al., 2006; Dowling et al., 2021; Rodriguez et al., 2020).

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In Western Europe, the intercropping of winter oilseed rape (OSR; *Brassica napus* L.) grown as cash crop with a frost-sensitive legume SP has been recently studied and is known to contribute to weed control (Cadoux et al., 2015; Dayoub et al., 2022; Lorin et al., 2015; Verret et al., 2017a). Verret et al. (2017a) also studied the effect of pure non-legume frost-sensitive SPs intercropped with OSR. They observed a high level of weed control but also a strong negative impact on OSR biomass and a yield significantly decreased by 600 kg ha^{-1}.

In contrast, legume SPs can improve OSR nitrogen nutrition, allowing for a fertilisation reduction of up to 30 kg N ha^{-1} without yield loss or with a slight yield increase (Lorin et al., 2016; Verret et al., 2017a). This N supply service depends on the legume SP species (Lorin et al., 2016). Among legumes, faba bean was found to have a particularly good potential for nitrogen supply (Verret et al., 2017a). Species' ability to accumulate N, their C:N ratio, and growing habits are the main factors explaining these differences (Lorin et al., 2017, 2016). The importance of the C:N ratio for the subsequent N supply of plant materials is also well-known in cover crops and crop residues (Finney et al., 2016; Justes et al., 2009; Nicolardot et al., 2001).

Therefore, OSR-SP intercropping has a great potential to reduce the quantity of synthetic N fertiliser and herbicide applied to OSR, which is high for this crop (de Vries et al., 2010; Rathke et al., 2005; Valantin-Morison and Meynard, 2008). During the last decade, farmers started to adopt this practice in Western Europe. For instance, in Switzerland, this method represented 8% of the OSR grown in the Canton of Vaud and approximately 20% in the Geneva Canton in 2018, according to the extension offices of these Cantons. More particularly, Swiss farmers grow complex OSR-SP species mixtures, which include four to ten SP species. These mixtures also include non-legume SP species such as buckwheat or niger (Baux and Schumacher, 2019). By mixing several SP species, farmers and advisors aim i) to achieve a high level of weed control, together with nitrogen provision, and ii) to increase service provision stability in adverse growing conditions. However, the main studies focused on two-component intercropping or rather simple SP mixtures (Lorin et al., 2015, 2016; Verret et al., 2017a). Therefore, we lack the knowledge to understand the effect of complex mixture composition on the intercropping potential to provide ecosystem services.

In this study, we addressed whether all species included in complex SP mixtures intercropped with OSR matter. Thus, we have chosen four common legume SPs found in Swiss multispecific mixtures intercropped with OSR (Baux and Schumacher, 2019). We conducted two simultaneous and complementary experiments focusing on OSR intercropped with a legume SP mixture made of berseem clover (*Trifolium alexandrinum* L.), grass pea (*Lathyrus sativus* L.), lentil (*Lens culinaris* Medik.), and common vetch (*Vicia sativa* L.). We investigated whether legume species included in SP mixtures intercropped with OSR contribute equally to shape intercropping performance in terms of crop biomass, ground cover, and plants' N acquisition. In the first experiment, we hypothesised that removing a legume species from a complex SP mixture may significantly affect the ability of the SP mixture to provide N supply or weed control, and we aimed to assess the weight of each species in this result. In parallel with the second experiment, we investigated the effect of adding a non-legume to the legume SP mixture on its biomass, its efficiency in competing against weeds, and its potential negative effect on OSR biomass and N status. Two non-legume species commonly found in the Swiss mixtures were tested: niger (*Guizotia abyssinica* (L.f) Cass) and buckwheat (*Fagopyrum esculentum* Moench). In this second experiment, we also investigated the potential role of mustard (*Sinapis alba* L.) and a legume, the faba bean (*Vicia faba* L. ssp. *minor*), when added in SP mixtures intercropped with OSR, as this species is missing in the Swiss mixtures.

2. Materials and methods

2.1. Study sites and pedoclimatic conditions

Field experiments were carried out in two contrasting locations in Western Switzerland. The experiments were undertaken on a basic loam or clay loam soil of the agronomic research station of Agroscope in Prangins in 2018 and 2019 (respectively, Table 1) and in a sandy loam soil of the Grange-Verney domain in Moudon in 2018 (Table 1). In Prangins, the previous crop was wheat, harvested in July for both years. Then, the field was cultivated twice at 8 cm and 15 cm depths to remove stubble, cereal volunteers, and weeds using a sweep cultivator (Kerner galaxis G300, Aislingen, Germany). The seedbed preparation was done using a rotary hoe (Alpego RH300, Lonigo, Italy). For the site of Moudon, the pre-crop was a pasture that was destroyed by ploughing, and then the seedbed was prepared using a rotary hoe. These practices are representative of those of the current Swiss oilseed rape (OSR) growers.

Data relative to temperatures and rainfall were gathered thanks to the meteorological stations of both experimental domains (Fig. 1; Agrometeo, 2021). In 2018, at both sites, the fall growing conditions were very dry. Over the growing period, only 40% and 13% of the 15-year average rainfall between 2006 and 2020 were recorded for Moudon and Prangins, respectively. In 2018 the site of Prangins was irrigated twice with a total amount of 30 mm of water, which allowed crop growth despite the very dry conditions of summer and fall of 2018. In Moudon, irrigation was not carried out. At this site, the plant growth was strongly limited by water deficiency, enhanced by the sandy loam soil texture. For Prangins 2019, the weather conditions were close to the local climatic references (Fig. 1).

2.2. Experimental design

Different service plant (SP) mixtures intercropped with OSR following an additive design were compared. Each year, the same design was repeated at each site and used to conduct two simultaneous and complementary experiments (Experiment 4L^{-}, and Experiment 4L^{+}). Both experiments were arranged in a single trial of three randomised complete blocks in each site (see supplementary materials). In each treatment, OSR and SPs were sown separately on the same day. First, the SPs were sown with a row spacing of 15 cm using a disc seeder (Great plains 3P1006NT, Marigny-les-Usages, France). For all mixtures, the sowing density of each SP species was equal to its recommended pure density as a cover crop divided by the number of species in the SP mixture. The recommended density of each SP species was based on local references (see supplementary materials). Then, the OSR (cv. Avatar) was sown at a density of 55 seeds per m^2 in Prangins or 50 seeds

Table 1
Sites description.

	Moudon 2018	Prangins 2018	Prangins 2019
Location	46°40′45. N 6°48′5	46°23′45. N 6°15′3	46°24′04. N 6°15′2
Altitude	514 m	384 m	408 m
Pre-crop	Pasture	wheat	wheat
Tillage	Ploughing	Cultivator 15 cm	Cultivator 15 cm
Sowing date	22.08.2018	20.08.2018	26.08.2019
Fall sampling date	23.10.2018	16.10.2018	29.10.2019
% Clay	11.8	22.1	27.1
% Silt	18.1	43.2	45.4
% Sand	70.1	34.7	27.5
%Organic matter	2.14	3.02	3.18
pH_{H2O}	6.5	8.1	8.0
Active $CaCO_3$ (%)	0.0	2.2	2.7
P_{Olsen} (‰)	0.08	0.05	0.04
$K_2O_{exchangeable}$ (‰)	0.20	0.32	0.17
CEC (me kg^{-1})	104	119	124

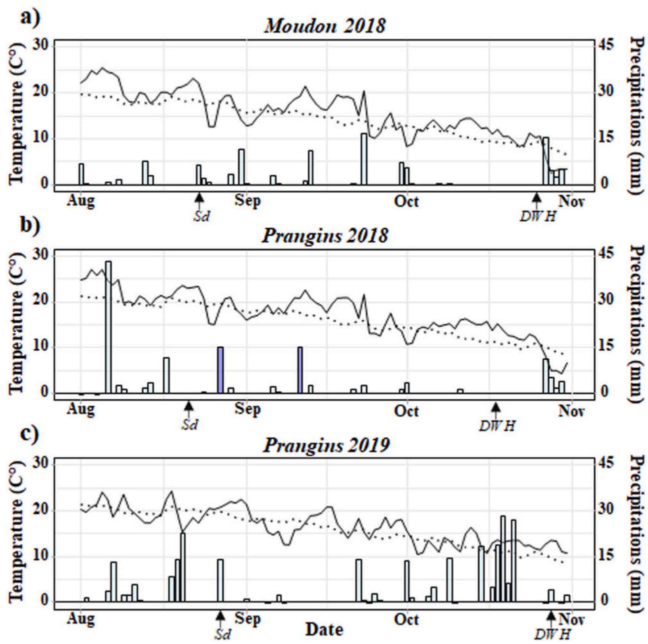

Fig. 1. Meteorological information of the experimental sites. Sd: Sowing date, DW H: biomass plant-sampling day. The black full line is the daily mean temperature in °C. The black dashed line is the daily mean temperature between 2006 and 2020. The light blue bars represent the rainfall in mm, and the blue bars represent the irrigation in mm.

per m^2 in Moudon, with a row spacing of 50 cm (Monosem, Largeasse, France). The sowing operations were done using a GPS-RTK system (John Deere, Moline, IL, USA). Each plot was 6 × 6 m. All the plant sampling and measurements were made at least 50 cm from the plot side.

2.2.1. Experiment 4L$^-$: Removal of a legume from a service plant mixture

Experiment 4L$^-$ consisted of removing one legume species from a four-legume SP mixture (4L) intercropped with OSR. The 4L treatment was made of berseem clover (cv. Winner), grass pea (cv. Merkur), lentil (cv. Red flash), and common vetch (cv. Omiros). We, thus, obtained four treatments (Fig. 2): i) 4L without clover (4L−C), ii) 4L without grass pea (4L−G), iii) 4L without lentil (4L−Le), and iv) 4L without vetch (4L−V).

2.2.2. Experiment 4L$^+$: Addition of a species to a service plant mixture

The Experiment 4L$^+$ was based on the same four legume reference mixtures (4L) intercropped to OSR. The treatments consisted of 4L, to which a fifth species was added. This species was either a legume [faba bean (cv. Fanfare)] or a non-legume [niger, buckwheat (cv. Lileja), or mustard (cv. Pirat)]. These four species were chosen based on their contrasting agronomic and functional traits (Tribouillois et al., 2015; Wendling et al., 2016). Thus, the modalities were (Fig. 2): i) 4L plus faba bean (4L+F), ii) 4L plus niger (4L+Ni), iii) 4L plus buckwheat (4L+B), and iv) 4L plus mustard (4L+M).

In addition to the eight treatments of both experiments and the 4L reference, pure OSR (R) was also grown as a second reference treatment (Fig. 2).

2.3. Plant and soil sampling and chemical analyses

The above-ground parts of OSR and SPs were sampled on a 1 m^2 plot before the first freezing in the second half of October: 23.10.2018 (890 °C d after sowing, base 0 °C), 16.10.2018 (989 °C d), and 29.10.2019 (995 °C d) for Moudon in 2018, Prangins in 2018 and in 2019, respectively (Table 1, Fig. 1). The weeds were sampled the same day on the same plot. In 2018, a subplot of 0.5 m^2 was used for this purpose. The collected SPs were sorted by species and fresh weighed.

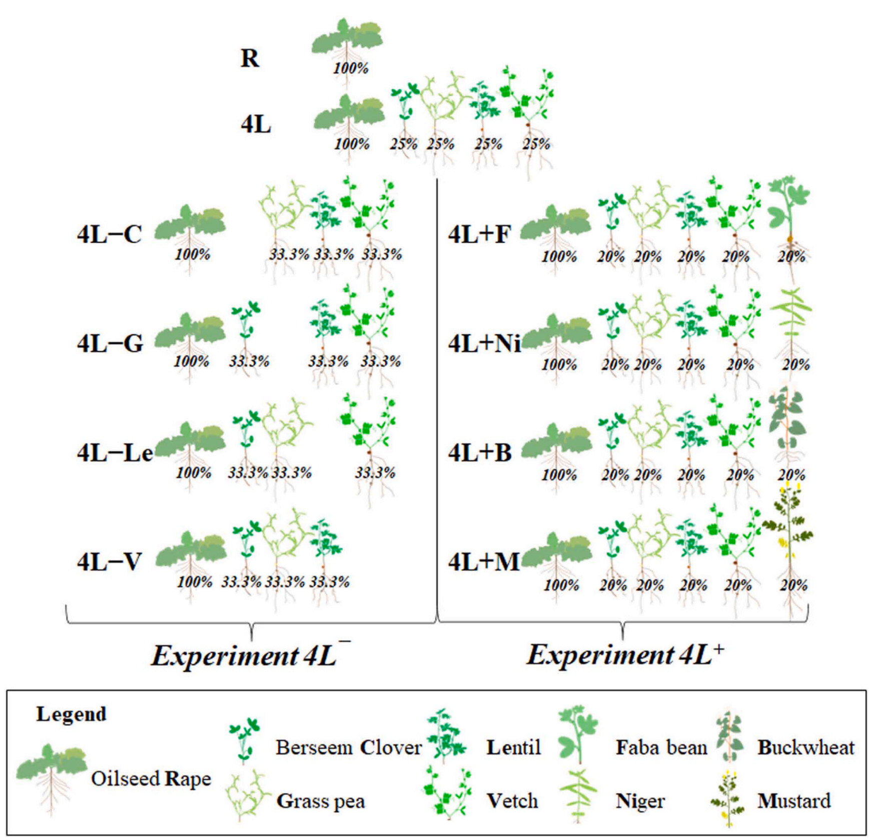

Fig. 2. Species composition of the treatments of Experiments 4L$^-$ and 4L$^+$. The percentage bellow each plant species is the proportion of the recommended seed density of the species (The sowing densities are presented in supplementary materials).

The four 4L legumes (clover, grass pea, lentil, and vetch) were pooled for dry weight measurements and chemical analysis. Excepted within the treatments 4L and 4L+F, where each species was dried in separate samples. The resulting plant samples (OSR, 4L SPs and weeds) were dried at 60 °C for 72 h and weighed. The dry samples were ground using a hammer mill (RETSCH SR 300, Haan, Germany) mounted with a 0.75-mm grid. Then, the plant N and C contents were measured using Dumas' method with a combustion analyser (ELEMENTAR Vario MAX cube, Hanau, Germany). The part of each species in the four legume mixture total dry weight was extrapolated based on the species' fresh weight and the fresh:dry weights ratio of each species that were measured within the 4L and 4L+F treatments.

After plant sampling, in each plot, soil samples were collected in the 0–30 cm and 30–60 cm layers. For each modality, the samples of the three blocks were pooled into a single sample. The soil mineral nitrogen (N-NO_3^- and N-NH_4^+) were KCl extracted and measured using colourimetry with a discrete analyser (THERMO FISHER SCIENTIFIC Gallery, Waltham, MA, USA). Before this analysis, the samples were stored below − 18 °C.

2.4. Ground cover assessment

The ground covered by plants was assessed on a 1 m^2 hand-weeded plot thanks to image analysis. Pictures were taken using a high-resolution digital mirrorless camera (CANON EOS M10, Tokyo, Japan) at a focal length of 15 mm using the kit lens (CANON EF-M 15–45 mm f/3.5–6.3, Tokyo, Japan). They were taken perpendicular to the ground using a tripod. The captor's distance to the ground was approximately 1.31 m. Then, all the pictures were cropped to a size of 0.5 × 1 m plot to ensure that we only took into account the weeded plot and to have a representative image sample of the field. This measurement was only carried out in Prangins.

Then, the image was analysed with a DeepLab machine learning model (Chen et al., 2018) that was parametrised on the image taken in this and similar experiments and showed 96.5% high accuracy (de Jong et al., 2022). This method allowed us to take into account the yellow leaves as well as SP flowers.

A set of pictures was produced in the OSR four-leaf stage on each weeded plot 30 (579 °Cd) and 32 (565 °Cd) days after sowing for in 2018 and 2019, respectively. On the plant biomass sampling date, a second set of pictures was made.

2.5. Statistical analysis

All data analyses were performed using R software (R Core Team, 2021). The plant dry weight, N content, and C:N ratio were analysed separately for each site and year. The ANOVAs always included block as a random factor thanks to the lme4 R package (Bates et al., 2015). Two-way ANOVAs were used to compare the year and treatment effect on ground covers in the Prangins site. For this analysis, the block was also considered as a random factor. For each ANOVA, the normality of residuals and homogeneity of variances were checked using Shapiro-Wilk and Bartlett tests and were visually controlled with quantile-quantile and residual plots. When the assumptions were not met, a log transformation was performed: the only transformed variable was the OSR dry weight in Prangins 2019, Experiment $4L^+$. The post-hoc tests used was Tukey HSD and Dunnett's test. Dunnett's test was used for each comparison of a treatment to a reference (R or 4L). In the other cases, we used the Tukey HSD. Both tests were performed using the emmeans and multcomp R packages (Hothorn et al., 2021; Lenth, 2021). For all tests, we considered a test with a *P*-value (*P*) lower than 0.05 to be significant.

In all figures and tables, points and numbers represent the means of the three replicates of a site, and the error bars or the numbers following the ± sign represent the standard deviations of the mean (SDs).

3. Results

3.1. Oilseed rape dry weight

The oilseed rape (OSR) dry weight was highly variable, ranging from 0.53 t ha^{-1} to 0.79 t ha^{-1} in Moudon ($P > 0.05$), 1.37 t ha^{-1} to 2.56 t ha^{-1} in Prangins 2018 ($P < 0.001$), and 0.88 t ha^{-1} to 2.56 t ha^{-1} in Prangins 2019 ($P < 0.001$; Table 2) and thus mainly depended on site and year. Significant OSR dry weight reductions occurred in intercropping compared to OSR grown alone in Prangins, ranging from − 47% to − 3% and − 66% to − 16%, in 2018 and 2019 respectively (Table 2).

When one service plant (SP) species was removed from the four legumes SP mixture (4L, made of berseem clover, grass pea, lentils, and common vetch), the dry weight of OSR did not significantly differ from the initial 4L treatment, except for the mixture without clover (4L−C) in Prangins 2018 ($P < 0.05$). The dry weight of OSR was only significantly reduced for 4L−C in Prangins both years, and 4L−V (4L without vetch) in 2019, compared to pure OSR (R; Table 2).

When mustard was added to 4L (4L+M) and, to a lesser extent, when buckwheat was included to the 4L mixture (4L+B), the OSR dry weight decreased compared to OSR grown alone (−29% to −47% in Prangins 2018 and −53% to −66% in Prangins 2019 for 4L+B and 4L+M, respectively; $P < 0.05$). Conversely, mixtures with legumes only (4L and 4L+F) had no significant impact on OSR dry weight compared to the OSR sole crop. Finally, the effects of niger addition to the 4L mixture (4L+Ni) on OSR dry weight were more impacted by year. In Prangins 2018, the OSR biomass of 4L+Ni was 34% and 31% lower than that of R or 4L, respectively ($P < 0.05$). In Prangins 2019, 4L+Ni had the highest OSR biomass among intercrops, with a 16% reduction of the OSR dry weight compared to pure OSR (R).

3.2. Dry weight of service plant mixtures

The dry biomass accumulated by SP mixtures also varied among sites and years. In Moudon, SP dry weights ranged from 0.09 t ha^{-1} for 4L−V to 0.26 t ha^{-1} for 4L+B. No significant difference was observed with the 4L reference that reached 0.15 t ha^{-1}. SPs accumulated more dry weight in Prangins, ranging from 0.26 t ha^{-1} and 0.36 t ha^{-1} for 4L−G to 1.23 t ha^{-1} and 1.62 t ha^{-1} for 4L+M in 2018 and 2019, respectively ($P < 0.05$; Table 2).

In Prangins, when a SP legume was removed, no difference was recorded among treatments: the biomass accumulated by the three-legume mixtures did not differ from the 4L. In these three-legume mixtures, the proportions of berseem clover, grass pea, and common vetch ranged from 23% to 51% (Fig. 3). Only lentil was underrepresented, with 15% to 28% of the total SP dry weight (Fig. 3). In Moudon, berseem clover failed to develop, representing 0% to 3.3% of the SP dry weight in the treatments in which it was included. None of the other species completely failed to develop.

Conversely, when a non-legume species was added to SP mixture, the recorded SP biomass varied over a wider range. Thus, adding buckwheat (4L+B) and mustard (4L+M) to the 4L mixture led to an increase in SP biomass in Prangins 2018 (0.89 t ha^{-1} and 1.23 t ha^{-1}, respectively) and Prangins 2019 (1.25 t ha^{-1} and 1.62 t ha^{-1}, respectively) compared to the other SP mixtures that reached a maximum of 0.44 t ha^{-1} and 0.62 t ha^{-1} during these two years ($P < 0.05$; Table 2). In fact, the non-legume species strongly dominated the other SP species in these two treatments. Buckwheat represented 69.3%, 73.0%, and 77.7% of the SP dry weight, and mustard represented 57.3%, 87.7% and 86.3% of the SP dry weight in Moudon 2018, Prangins 2018, and Prangins 2019, respectively (Fig. 3). Adding niger to the 4L mixture did not affect the total biomass accumulation. The niger share within SP dry weight mixtures varied strongly depending on growing conditions. It represented 45.3% of the 4L+Ni mixture in Prangins 2018 vs 17.0% in Prangins 2019 (Fig. 3; Table 2).

Table 2
Oilseed rape, service plant and weed dry weight across treatments and sites.

	Experiment 4L^{-}						
	R	4L	4L−C	4L−G	4L−Le	4L−V	*P*
OSR dry weight (t ha^{-1})							
Moudon 2018	0.79 ± 0.26	0.67 ± 0.07	0.60 ± 0.17	0.64 ± 0.10	0.62 ± 0.17	0.62 ± 0.12	ns
Prangins 2018	2.56 ± 0.32 a	2.49 ± 0.12 ab	1.79 ± 0.12 b	1.89 ± 0.43 ab	2.29 ± 0.36 ab	2.09 ± 0.14 ab	*
Prangins 2019	2.56 ± 0.46a	1.83 ± 0.18 ab	1.56 ± 0.38 b	1.98 ± 0.36 ab	2.07 ± 0.22 ab	1.68 ± 0.15 b	*
SP dry weight (t ha^{-1})							
Moudon 2018		0.15 ± 0.04	0.10 ± 0.05	0.14 ± 0.03	0.14 ± 0.05	0.09 ± 0.04	ns
Prangins 2018		0.35 ± 0.02 ab	0.44 ± 0.02 a	0.26 ± 0.04 b	0.30 ± 0.05 b	0.35 ± 0.05 ab	**
Prangins 2019		0.58 ± 0.13	0.50 ± 0.15	0.36 ± 0.26	0.48 ± 0.01	0.46 ± 0.19	ns
Weeds dry weight (t ha^{-1})							
Moudon 2018	0.25 ± 0.30	0.09 ± 0.03	0.16 ± 0.13	0.11 ± 0.11	0.04 ± 0.03	0.15 ± 0.15	ns
Prangins 2018	0.03 ± 0.02	0.16 ± 0.16	0.25 ± 0.19	0.18 ± 0.12	0.02 ± 0.01	0.12 ± 0.04	ns
Prangins 2019	0.12 ± 0.06	0.18 ± 0.10	0.04 ± 0.01	0.05 ± 0.03	0.09 ± 0.05	0.13 ± 0.12	ns

	Experiment 4L^{+}						
	R	4L	4L+F	4L+Ni	4L+B	4L+M	*P*
OSR dry weight (t ha^{-1})							
Moudon 2018	0.79 ± 0.26	0.67 ± 0.07	0.70 ± 0.19	0.77 ± 0.08	0.53 ± 0.09	0.62 ± 0.21	ns
Prangins 2018	2.56 ± 0.32 a	2.49 ± 0.12 a	2.48 ± 0.05 a	1.69 ± 0.29 b	1.83 ± 0.06 b	1.37 ± 0.26 b	***
Prangins 2019	2.56 ± 0.46 a	1.83 ± 0.18 abc	1.53 ± 0.17 bcd	2.15 ± 0.61ab	1.22 ± 0.11 cd	0.88 ± 0.16 d	***
SP dry weight (t ha^{-1})							
Moudon 2018		0.15 ± 0.04	0.16 ± 0.04	0.16 ± 0.05	0.26 ± 0.02	0.20 ± 0.10	ns
Prangins 2018		0.35 ± 0.02 b	0.34 ± 0.09 b	0.35 ± 0.15 b	0.89 ± 0.22 a	1.23 ± 0.29 a	***
Prangins 2019		0.58 ± 0.13 b	0.62 ± 0.12 b	0.37 ± 0.18 b	1.25 ± 0.21 a	1.62 ± 0.31 a	* **
Weeds dry weight (t ha^{-1})							
Moudon 2018	0.25 ± 0.30	0.09 ± 0.03	0.10 ± 0.05	0.06 ± 0.03	0.13 ± 0.09	0.09 ± 0.10	ns
Prangins 2018	0.03 ± 0.02	0.16 ± 0.16	0.15 ± 0.12	0.18 ± 0.23	0.10 ± 0.07	0.02 ± 0.02	ns
Prangins 2019	0.12 ± 0.06 ab	0.18 ± 0.1 a	0.05 ± 0.04 b	0.13 ± 0.03 ab	0.10 ± 0.05 ab	0.05 ± 0.02 b	*

Figures are the means of three replicates of a treatment in a site ± SD. Asterisks indicate the significance of the differences ns: $P > 0.05$, * : $P < 0.05$, * *: $P < 0.01$ and * ** : $P < 0.001$. Different letters: a, b, c and d indicate significant differences across treatments according to Tukey's HSD test.

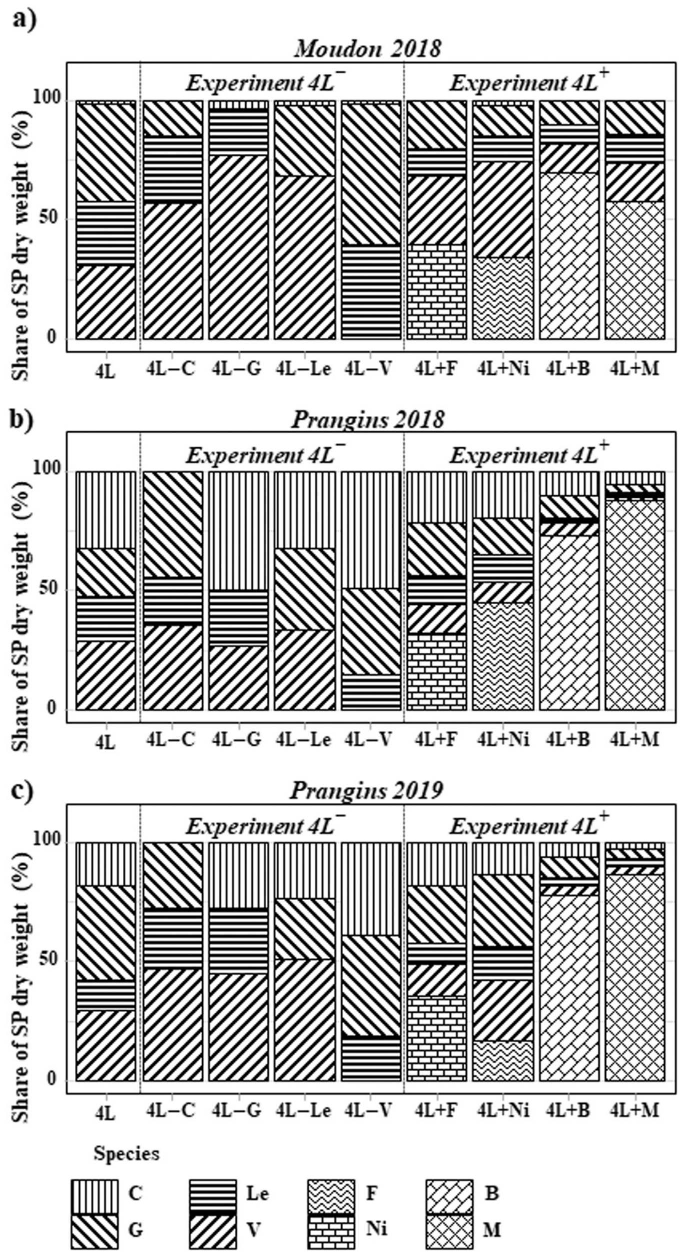

Fig. 3. Species composition of service plant mixtures dry biomass according to site (%). Share of SP (service plant) dry weight is the percentage of a given species within aboveground SP dry weight. The pattern represents the SP species. C: berseem clover, G: grass pea, Le: lentil, V: vetch, F: faba bean, Ni: niger, B: buckwheat and M: mustard.

3.3. Weed biomass

The weed dry biomass was globally low, especially in Prangins, where no significant differences were recorded between treatments over the two years, except between 4L+M and 4L in 2019 (Table 2). Thus, in Prangins, the weed biomass only accounted for 0% to 10% of the total biomass produced by the whole plant cover (Fig. 4). In Moudon, the weed dry weight proportions were higher, ranging from 6% to 24%. However, the variation coefficients of weed dry weights within treatments were 66%, 67%, and 78% on average in Moudon 2018, Prangins 2018 and Prangins 2019, respectively, which did not allow the comparison between treatments.

Contrasting trends appeared when comparing the OSR:SPs dominance ratio. Removing a legume species or adding faba bean or niger to a 4L mixture resulted in a low proportion of SPs in the total plant biomass (OSR+SP+weeds), it ranged from 11% to 28%, on average (Fig. 4). Conversely, the proportion of SPs in the total plant dry weight when adding mustard or buckwheat (4L+B, 4L+M) was much higher on average for a given site and year; it ranged from 28% to 48% and 22% to 58% for 4L+B and 4L+M, respectively (Fig. 4b).

3.4. Ground cover

The ground cover measured at the four-leaf stage of OSR showed no significant difference among mixtures made of legumes only (Fig. 5a). On average, OSR-legume mixture intercropping covered 44% to 53% and 46% to 52% of the soil in Prangins 2018 and 2019, respectively. OSR grown alone covered, on average, 42% and 43% of the soil in 2018 and 2019, respectively (Fig. 5a). 4L+F (4L with faba bean) and 4L+Ni (4L with niger) showed the same pattern (Fig. 5c). Both the mixtures with buckwheat and mustard (4L+B, 4L+M) had a higher soil cover level compared to pure OSR (R), with ground covers of 58% and 62%, and 64% and 66% in 2018 and 2019, respectively, at the four-leaf stage of OSR ($P < 0.05$; Fig. 5c). The treatment with mustard (4L+M) also covered the soil faster than the 4L reference ($P < 0.05$; Fig. 5c).

In late fall, adding a non-legume or faba bean to the 4L mixture had no significant impact on the ground cover ($P > 0.05$; Fig. 5d). These treatments did not significantly improve ground cover compared to pure OSR (R) on the fall sampling day. Conversely, adding legume-based SP globally improved ground cover compared to OSR grown alone (R). These differences were higher and significant in the 4L and 4L−C treatments, with average gains of 10% and 8%, respectively, compared to the pure OSR (R). Regardless of the year effect, the other three legume mixtures also tended to increase the ground cover compared to R according to the Dunnett post hoc test with *P*-values of 0.04, 0.06 and 0.11 for 4L−G (4L without grass pea), 4L−Le (4L without lentil) and 4L−V (4L without vetch), respectively.

3.5. Plant N accumulation

In Prangins, OSR N accumulation ranged between 26.9 kg N ha^{-1} and 54.8 kg N ha^{-1} in 2018 and 30.1 kg N ha^{-1} to 66.5 kg N ha^{-1} in 2019 ($P < 0.05$). It strongly differed between the experimental sites. In Moudon, the amount of N ranged from 21.1 kg N ha^{-1} to 28.5 kg N ha^{-1} in the OSR ($P > 0.05$). In all cases, intercropping did not significantly increase the OSR N compared to the pure stand (R) (points lower or close to 0 on the y-axis of Fig. 6). The OSR N amount was not significantly impacted compared to the 4L reference when a legume was removed from the SP mixture, except for 4L−C in Prangins 2018 ($P < 0.05$). The addition of any of the three non-legumes decreased the OSR N compared to 4L in Prangins 2018 ($P < 0.05$, $P < 0.1$, $P < 0.01$ for 4L+Ni, 4L+B and 4L+M, respectively). In 2019, only the 4L+M treatment showed a lower OSR N content than the 4L treatment ($P < 0.05$).

The SPs accumulated 9.9 kg N ha^{-1} to 25.3 kg N ha^{-1} and 12.7 kg N ha^{-1} to 42.2 kg N ha^{-1} in Prangins 2018 and 2019, respectively, and 4.1 kg N ha^{-1} to 8.0 kg N ha^{-1} in Moudon (Fig. 6). Neither the removal of a legume nor the addition of faba bean to the four legume mixtures led to any significant difference in N accumulation by SPs compared to the 4L treatment ($P > 0.05$; Fig. 6). This was found at any site except for the 4L−C mixture in Prangins 2018, which accumulated more N in SP biomass than the 4L reference mixture ($P < 0.05$; Fig. 6b). The 4L+M treatment was the only mixture that accumulated more N in SPs than 4L (11.8 kg N ha^{-1} and 19.3 kg N ha^{-1} more in Prangins 2018 and 2019, respectively; $P < 0.05$; Fig. 6e, f). The addition of niger had the opposite effect in Prangins 2019 and tended to decrease the N accumulated by SPs by 10.1 kg ha^{-1} ($P < 0.1$).

The total N amount accumulated by the cover (OSR+SPs) was always higher or close to the pure OSR N amount (no point significantly lower than the 1:−1 grey dashed line of Fig. 6). The effect of legume SP mixture was only significant in in Prangins 2018 (Fig. 6b), where the 4L

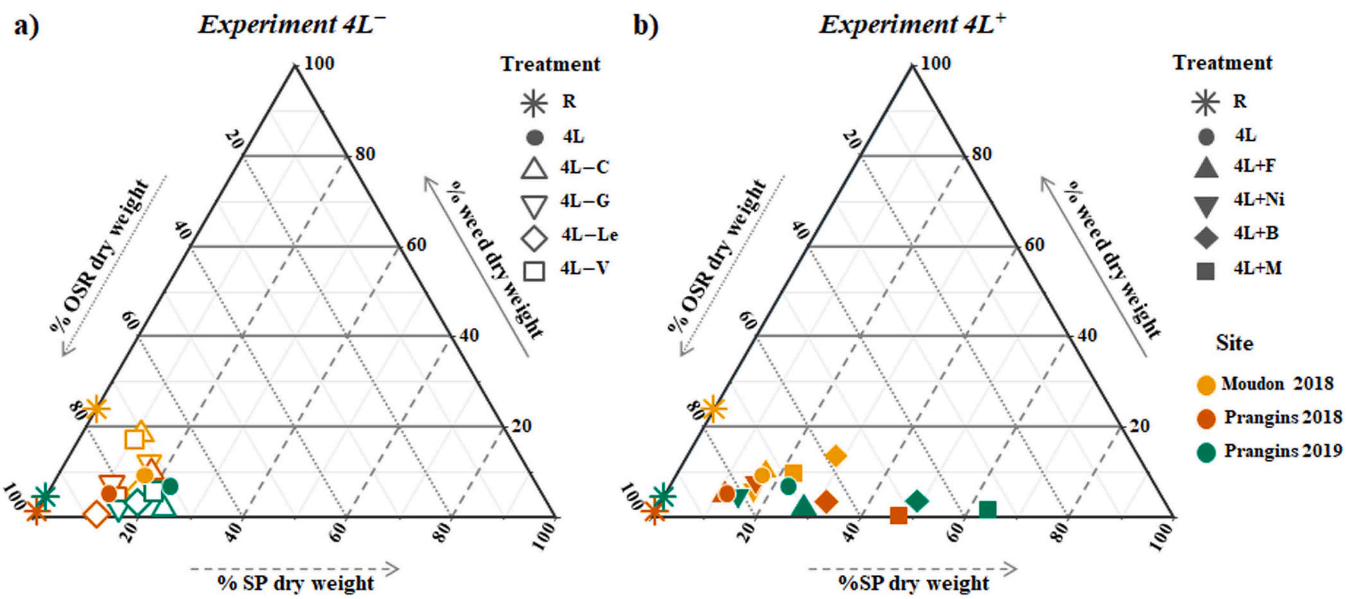

Fig. 4. Proportion of the biomass of oilseed rape, service plants and weed dry weights according to site and SP mixtures. % OSR dry weight: is the proportion of oilseed rape (OSR) in the total aboveground dry weight of plant, % SP dry weight: is the proportion of service plant (SP) in the total aboveground dry weight of plant and % weeds dry weight: is the proportion of weed in the total aboveground dry weight of plant. Each point represents the mean of the three replicates of a treatment in a site.

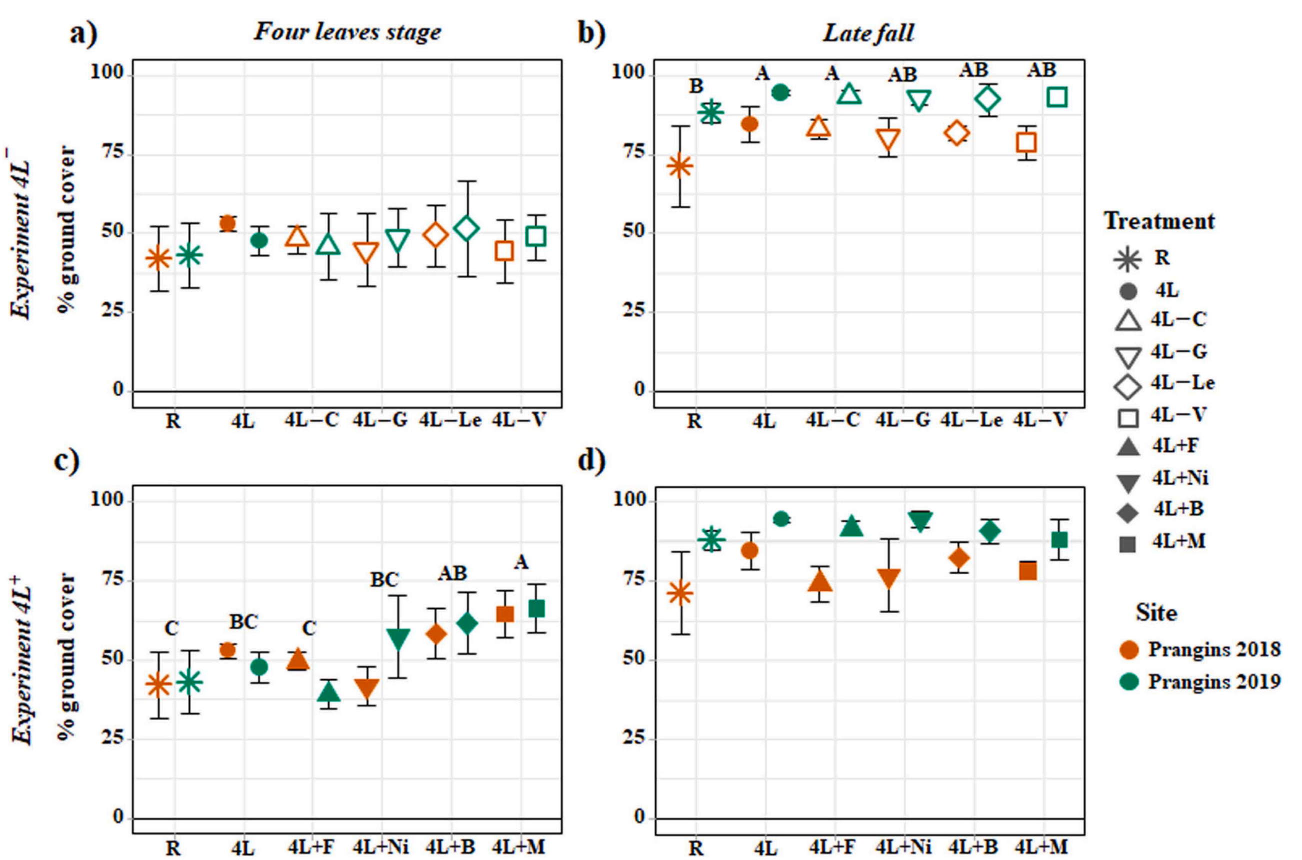

Fig. 5. Proportion of ground covered by the different service plant mixture-oilseed rape intercropping in early (a), (c) and late fall (b), (d). ANOVAs were performed to test the treatment effect on the ground cover, and the year and the block were added as random factors. $P > 0.05$ for (a) and (d), $P < 0.001$ for (c) and $P < 0.05$ for (b). The letters A, B and C represent the groups that significantly differ according to a Tukey HSD test. The points represent the mean ground cover of the three replicates of a site in %, and the error bars represent the SD.

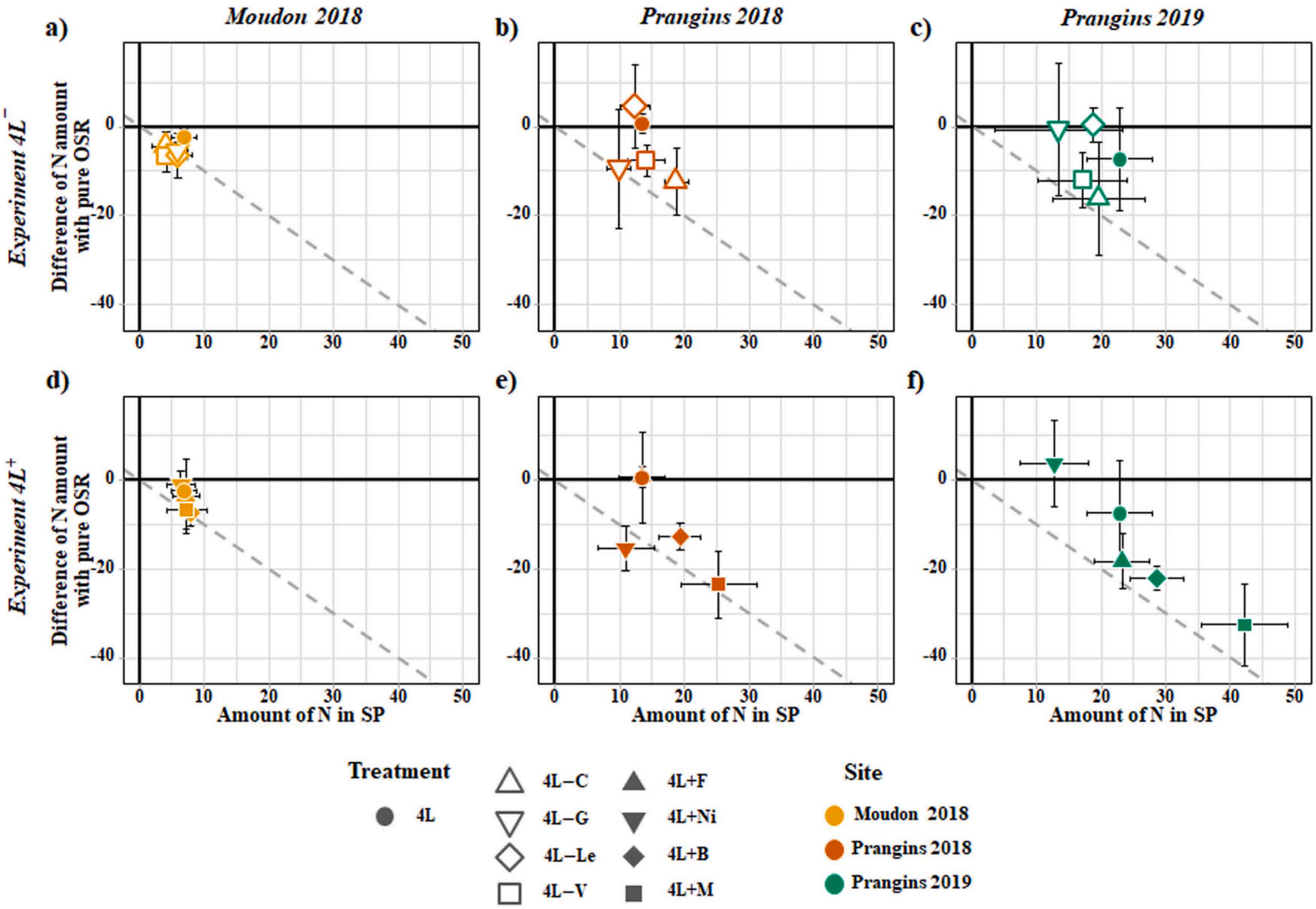

Fig. 6. **:** Impact of the mixture on oilseed rape and service plant N accumulation in aboveground parts (kg N ha^{-1}). The points represent the means of three replicates of a site. The vertical error bars represent the variability of N amount in OSR within a treatment. The horizontal error bars show the variability in the N amount in SPs for each treatment. The dashed grey line represents the limit below which the total amount of N accumulated by the cover is lower than the average N amount in the pure rapeseed.

and 4L−Le treatments accumulated 14.1 kg N ha^{-1} and 16.9 kg N ha^{-1} more than the pure OSR, respectively, in their plant cover (OSR+SPs, $P < 0.05$). The treatment without grass pea (4L−G) accumulated the lowest amount of N among intercrops, with a total of 50.8 kg N ha^{-1} (significantly less than 4L; $P < 0.05$). The addition of non-legume to the SP mixture had a greater impact on SP N in Prangins 2018 and 2019. The effect of niger was year dependent, leading to a global decrease of the N amount accumulated by the plant cover (N OSR+ N SPs) of − 18.5 kg N ha^{-1} less than that in the 4L treatment in Prangins 2018 ($P < 0.05$; Fig. 6e); this difference was only − 0.6 kg N ha^{-1} in Prangins 2019 ($P > 0.05$; Fig. 6f).

The soil mineral nitrogen in the 0–60 cm horizon did not vary among treatments but was highly dependent on the site: less than 6 kg ha^{-1} in Moudon, 40 kg ha^{-1} to 52 kg ha^{-1} in Prangins 2018 and 18 kg ha^{-1} to 27 kg ha^{-1} in Prangins 2019.

3.6. C:N ratio of service plants

Removing one legume species or adding faba bean to a 4L mixture did not modify the C:N ratios, regardless of the site and year (Table 3).

Conversely, adding a non-legume species to the 4L legume mixture showed more variable results. Mustard (in Prangins 2018 and 2019) and buckwheat (regardless of the site and year) led to a significant increase in the C:N ratio (Table 3). In 4L+B, the C:N ratios were 14.8, 20.7, and 19.5 in Moudon, Prangins 2018, and 2019, respectively, while they were 12.4, 21.3, and 17.0 for 4L+M (Table 3).

In the 4L treatment, the C:N ratio varied among species: it was higher in the clover than in the grass pea, with ratios of 12.9 vs 11.0 in Prangins 2018 and 12.9 vs 10.0 in Prangins 2019 ($P < 0.01$, see supplementary materials). Among the added species, buckwheat had the highest C:N ratio, with values of 17.6, 27.6, and 25.1 in Moudon, Prangins 2018, and 2019, respectively ($P < 0.05$). Faba beans reached values close to those of vetch and lentil. Finally, niger and mustard were intermediate between faba bean and buckwheat (see supplementary materials).

Table 3
C:N ratio of service plant mixtures.

	Experiment $4L^-$					
	4L	4L−C	4L−G	4L−Le	4L−V	*P*
Moudon 2018	10.6 ± 0.3	11.0 ± 0.5	11.1 ± 0.5	10.8 ± 0.2	10.4 ± 0.5	ns
Prangins 2018	12.1 ± 0.3	10.9 ± 0.6	12.3 ± 0.8	11.4 ± 0.1	11.7 ± 0.8	.
Prangins 2019	10.7 ± 0.3	10.8 ± 0.1	11.2 ± 0.7	11.2 ± 0.5	11.2 ± 1.2	ns
	Experiment $4L^+$					
	4L	4L+F	4L+Ni	4L+B	4L+M	*P*
Moudon 2018	10.6 ± 0.3b	10.7 ± 0.7b	11.5 ± 0.3b	14.8 ± 1.7a	12.4 ± 0.9ab	**
Prangins 2018	12.1 ± 0.3b	11.8 ± 0.1b	14.4 ± 2.4b	20.7 ± 2.1a	21.3 ± 0.3a	***
Prangins 2019	10.7 ± 0.3b	11.6 ± 0.5b	11.9 ± 2.1b	19.5 ± 1.6a	17.0 ± 3.1a	***

Figures are the means of three replicates of a treatment in a site ± SD. Asterisks indicate the significance of the differences ns: $P > 0.1$,.: $P < 0.1$, * : $P < 0.05$, **: $P < 0.01$ and *** : $P < 0.001$. Different letters: a and b indicate significant differences across treatments according to Tukey's HSD test.

4. Discussion

4.1. Effect of the legume service plant species composition on intercropping growth

Removing or adding a species to a legume service plant (SP) mixture had a limited impact on oilseed rape (OSR) or SP dry weight and on the amount of N. This result was surprising, as these legumes have contrasting traits, dry weigh accumulation and N contents when grown in pure stands or intercropped with OSR (Büchi et al., 2015; Dayoub et al., 2017; Guinet et al., 2018; Tribouillois et al., 2015; Wendling et al., 2016; Couëdel et al., 2018; Jeromela et al., 2017).

The mixtures with legumes also had a non-significant impact on the early ground cover. They achieved (or tended to achieve) higher levels of soil cover than sole OSR in the late fall. However, at this time, there was no difference among legume mixtures. This is consistent with Elhakeem et al. (2021), who observed no differences in ground cover dynamics among pure stands of vetch, berseem clover, or faba bean and reported that legumes cover the soil later than non-legumes. Our results confirm the hypothesis that mixtures of legume SPs contribute to improving the ground cover but only in the late stage, which can limit their potential capacity to control weeds. The reviews of Osipitan et al. (2018) and Gerhards and Schappert (2020) reported no effect of the species richness of cover crop mixtures on weed suppression, which is consistent with our results and considering that quick soil cover has been reported to enhance weed control (Brennan and Smith, 2005; Dorn et al., 2015).

A closer look at the total quantity of N accumulated by the plants shows that the four legumes mixture (4L) and the treatment without lentil (4L−Le) had the highest N accumulation in OSR+SPs in Prangins 2018, thanks to a lesser decrease of N accumulation in OSR, compared to pure OSR (Fig. 6). This finding also emphasises that niche complementarity between legume and OSR is a key process in explaining potential benefits from OSR-SPs intercropping, although, the OSR dominated the plant dry weight of all treatments that included only legumes SP in our experiments (Fig. 3). This distribution is, however, consistent with the literature, as legumes are known to be dominated in mixtures with non-legumes, especially when N is not limiting (Baraibar et al., 2020; Brennan et al., 2011; Lawson et al., 2015).

The N contents of OSR and SPs are not the only variables to consider for evaluating the potential N supply service. The C:N ratio is a major indicator that must be carefully examined due to its impact on net N mineralisation (Finney et al., 2016; Justes et al., 2009). Wendling et al. (2016) showed that different legume species could have very contrasting C:N ratios. We also observed these differences among species within the mixtures. Berseem clover had the highest C:N ratio, which is consistent with Couëdel et al. (2018). These differences were diluted in the mixture, resulting in a stable C:N ratio across the different treatments that only included legume species.

4.2. Effect of the inclusion of a non-legume in service plant mixtures on oilseed rape and service plants biomasses

Dominant non-legume species, even at low density, strongly modified the trade-off between the reduction of OSR growth and services provision. In our experiment, non-legume SPs were integrated into mixtures with legumes at one-fifth of their recommended sowing density. Therefore, these species competed with both OSR and legume SPs. In our study, the various non-legume SPs behaved differently. Mustard and buckwheat reduced the biomass and N accumulation of the OSR and the other SPs (legumes) compared to the other treatments. Consequently, the addition of these species did not lead to a significant increase in the total plant community (OSR+SPs) dry weight or N accumulation. The introduction of buckwheat and, to a lesser extent, mustard also impacted the potential of subsequent N supply after the death of SPs by increasing the C:N ratio of the SP mixture.

However, these mixtures covered the soil faster in the early stage, which is critical for competition against weeds (Riemens et al., 2022). These results are consistent with Verret et al. (2017a), who showed a strong negative impact of pure non-legume SPs on both OSR and weeds.

Niger greatly differed from the two other non-legumes. This species had a slow growth in early stages and its growth was highly variable across the experimental site or year. In all sites, the mixture made of four legumes SP and niger (4L+Ni) had the lowest amount of N accumulated in SPs among the five SP mixtures treatments (Experiment $4L^+$, Fig. 6). For this treatment, the total N accumulated by the plant community (OSR+SP) ranged from the highest to the lowest between 2018 and 2019 in Prangins (Fig. 6). Wendling et al. (2019) also stated that niger was more sensitive to growing conditions than other species. In our experiments, this sensitivity was not an asset in terms of the potential to provide services. However, further study of service plants' sensitivity to growing conditions could contribute to developing SP mixtures that provide services in favourable conditions and avoid disservices in less favourable growing conditions.

4.3. Functional redundancy, dilution and dominance effects are key processes in designing service plant mixtures

Our experiments can help to draw general principles for further research on highly diversified intercrops involving cash crops and for mixture design procedures of practitioners. First, adding or removing a legume from the four legumes mixture (4L) mixture had little impact on variables of interest. This suggests that the complementarity between legume species already reached its maximum potential when a mixture of three legumes was intercropped with OSR in Moudon 2018 and Prangins 2019 (or was close to being reached in Prangins 2018). Consequently, introducing an additional legume species may lead to functional redundancy. The low density of each species within complex mixtures (dilution) could also explain this similarity. For example, the C:

N ratios of the various legume species differed if taken individually, but the average C:N ratio remained similar across legume mixtures.

Adding a dominant non-legume species, such as mustard, led to a strong reduction in legume SPs and OSR biomass. Even if mustard exhibits traits close to those of OSR, the depressive effect on legume dry weight was strong. This effect could be explained by the sowing pattern (mixture of legumes and mustard sown on the same line and separate from OSR lines), as legume dry weight is often more impacted when they are intercropped in the same row as non-legumes (Cheriere et al., 2020, 2023; Stefan et al., 2022). To a lesser extent than the dominant species, the addition of faba bean represented a higher part of the dry biomass of SPs (40% to 33% depending on site, Fig. 3) than its sown density (20%, Fig. 2), which is consistent with the fact that this species is less sensitive to competition than other species, such as grass pea (Bousselin et al., 2021). It could be explained by its capacity to grow faster than other legumes, to reach high levels of N_2 fixation when grown with other species and to enhance soil microbial activity when intercropped with OSR (Bousselin et al., 2021; Dayoub et al., 2017; Drut et al., 2023;Verret et al., 2017a). However, this species did not modify the C:N ratio of the SP mixture. The lack of influence of the faba bean could be explained by its low relative sowing density. Unlike non-legume species, a strong dilution effect prevented the faba bean from shaping the cover traits and providing higher levels of ecosystem services, as observed by Verret et al. (2017a) when it was used as a single service plant. The recent work of Bybee-Finley et al.(2022) on cover crops mixtures highlighted evidence that reducing the seedling rate of dominant species and maintaining the seedling rate of less competitive species could be a way to mitigate the effect of interspecific interactions within complex mixtures.

4.4. Focus on the effect of the growing conditions

These results must be interpreted considering the contrasting growing conditions of the different site-year. In Moudon, the very dry conditions due to the low rainfall and the sandy loam soil texture, along with low N availability, limited the growth of both OSR and SPs. In this case, the specific diversity did not allow us to overcome the growing condition constraints. Intercropping is often reported as a good way to mitigate adverse growing conditions (Grange et al., 2021; Wendling et al., 2019; Bybee-Finley et al., 2016), but this effect remains limited when the growing conditions are too difficult or when complementarity between species is lacking (Baraibar et al., 2018; Zhang et al., 2019). In their review, Loreau et al. (2001) also observed that the effect of diversity on biomass production was smaller in unfavourable environments.

Indeed, the dry weight of SP species within the mixture was often different from its initial sowing density (Fig. 3). The species growth depended on the site, which is consistent with Baraibar et al. (2020), Stefan et al. (2021) and Schöb et al. (2023) who demonstrated that cover crop mixture species composition was highly dependent on growing conditions. For example, the clover did not emerge in Moudon. At this site, the common vetch represented 57% to 77% of SP dry weight in the mixtures with three legumes species (4L−C, 4L−G and 4L−Le; Fig. 3). Therefore, both three-legume and four-legume treatments achieved similar SP dry weights despite the clover failing to develop.

The growing conditions were much more favourable at the Prangins site in both years. The dry weight of plants remained lower than reported in the literature; for example, in the experiment of Verret et al. (2017a), the dry weight of legume SPs reached 0.81 t ha^{-1}. After plant sampling, in Prangins, we measured a higher amount of residual nitrogen in 2018 than in 2019, which could explain the higher relative share of OSR in the total plant biomass in 2018 (Fig. 4). Lorin et al. (2015) observed that OSR overtook legume SPs in N-fertilised plots. This finding is consistent with the fact that the benefits of diversity are maximal when N availability or other growing conditions are limiting but not drastically unfavourable (Corre-Hellou et al., 2006; Gaudin et al., 2014; Loreau et al., 2001).

5. Conclusion

Based on our experiments, we can state that adding a service plant species within a complex mixture can strongly modify the service plants biomass, its composition (C:N ratio) and its impact on oilseed rape growth. This effect was particularly true for buckwheat and mustard, which had very contrasting traits compared to the service plant species of our four-legume reference mixture (with berseem clover, grass pea, lentil, and common vetch). The impact of the addition or subtraction of a legume was much smaller and had little, if any, impact on oilseed rape and service plants growth. More investigations are needed to better understand the effect of growing conditions on plant-plant and plant-soil interactions. Indeed, growing conditions remain a key driver of plant growth, which is essential to allow the expression of species complementarity.

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CRediT authorship contribution statement

Baux Alice: Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Writing – review & editing. **Bousselin Xavier:** Conceptualization, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing, Methodology. **Valantin-Morison Muriel:** Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Writing – review & editing. **Cassagne Nathalie:** Writing – review & editing. **Fustec Joëlle:** Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Writing – review & editing. **Lorin Mathieu:** Investigation, Methodology, Supervision, Writing – review & editing, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. UFA Samen, Florin, and Nutriswiss were not involved in the study design, data collection, analysis, and interpretation of data, nor were they involved in writing the report and decision to submit the article for publication.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eja.2024.127097.

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