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Author(s):

Engbersen, Nadine ; Stefan, Laura; Brooker, Rob W.; Schöb, Christian 

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RESEARCH ARTICLE

Temporal dynamics of biodiversity effects and light-use-related traits in two intercropping systems

Nadine Engbersen¹  | Laura Stefan¹  | Rob W. Brooker² | Christian Schöb¹ 

¹Department of Environmental Systems Science, Institute of Agricultural Sciences, ETH Zurich, Zürich, Switzerland

²Ecological Sciences, The James Hutton Institute, Aberdeen, UK

Correspondence

Nadine Engbersen, Department of Environmental Systems Science, Institute of Agricultural Sciences, ETH Zurich, Universitätstrasse 2, 8092 Zürich, Switzerland.
Email: nadine.engbersen@usys.ethz.ch

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Abstract

Introduction: Intercropping systems can be more productive than their respective monocultures and this positive net biodiversity effect is caused by complementarity and selection effects. While the complementarity effect is caused through resource partitioning or facilitation, the selection effect operates via the greater probability that a more diverse community contains a dominant and high-yielding species which will account for the majority of productivity in that community. Here, we investigated how light-use-related traits contribute to the net biodiversity effect via complementarity or selection effects and how these relationships change throughout an annual growing season.

Materials and Methods: We conducted weekly destructive harvests to examine temporal dynamics of biodiversity effects in two crop mixtures (oat–lupin and oat–camelina) and their respective monocultures. We linked the biodiversity effects to traits related to light use (i.e., light interception, plant height, photosynthetic efficiency and photosynthetic capacity) and investigated how these relationships changed over time.

Results: We found that the net biodiversity and selection effect increased over time in both mixtures, while complementarity effects increased only in the oat–lupin mixture. More intercepted light and taller plants in mixtures compared to monocultures positively contributed to biodiversity effects in both mixtures. Strategies for shade tolerance differed between the mixtures, that is, increased photosynthetic capacity and increased photosynthetic efficiency contributed to a positive net biodiversity effect in the oat–lupin and oat–camelina mixture, respectively.

Conclusion: By linking the temporal dynamics of the net biodiversity effect and its two additive components to light-use-related traits in two different crop mixtures, this study demonstrates that complementary light use contributes to overyielding in intercropping systems. Such understanding is important for the design of effective intercropping systems and developing new crop cultivars suited to these environments.

KEYWORDS

biodiversity effects, intercropping, light use, plant traits, temporal dynamics

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1 | INTRODUCTION

In modern agriculture, high productivity often comes at the price of sustainability.¹ A key strategy to implement sustainable agriculture is to restore on-farm biodiversity through diversified farming systems.² One route to increase biodiversity in agricultural systems is intercropping, where at least two crop species are cultivated on the same field at the same time. Intercropping aims to sustainably increase yields through benefits such as improved resource capture and lower artificial inputs.³ Resource partitioning is considered a driving force for positive biodiversity effects in diverse plant communities.⁴ This resource partitioning can occur above- and belowground and minimizes the niche overlap between species and thus enables an increased resource capture in the crop mixture compared to the monoculture. While many studies have observed partitioning of belowground resources,^{5,6} evidence that these processes contribute to positive biodiversity effects remains limited.⁷ This suggests that complementary use of light might be an important, but to-date, overlooked mechanism driving increased productivity in diverse communities.^{8,9}

Crop mixtures are known to be more efficient at intercepting light compared to monocultures, which is due to complementary use of aboveground space when intercropped species differ in their aerial architecture and thus create more complex canopies that can intercept more light.^{10,11} The increased light interception in crop mixtures comes at the cost of shading, where shorter crops suffer shading from taller crops.¹² As shading is omnipresent in nature, plants have adapted to tolerate shade and have developed different strategies to optimize carbon gain even under low light conditions. These adaptations encompass—among others—increased photosynthetic capacity¹³ or photosynthetic efficiency.¹⁴ Photosynthetic capacity describes the maximum rate at which a leaf is able to fix C and has been tightly associated with leaf N content.¹⁵ For instance, as a response to lower light conditions in the mixed cropping system, leaf N content in watermelon increased when cultivated in mixture compared to when cultivated in monoculture.¹³ Photosynthetic efficiency describes the efficiency by which captured light is converted into biomass.¹⁶ Photosynthetic processes are known to be highly sensitive to shading and plants can adapt their photosynthetic characteristics to various light environments,¹⁷ as shown in a recent study where increased efficiency of photosystem II (PS_{II}) in proso millet was observed in response to being grown in a mixture.¹⁴

Thanks to these adaptations, mixed cropping systems can enable positive light-driven biodiversity effects. In this study, we relate positive biodiversity effects mainly to the positive effect of increased plant species richness on plant primary productivity. However, the relative extent to which complementary use of light contributes to positive biodiversity effects in intercropping is poorly understood.¹⁸ Positive biodiversity effects are measured through the net biodiversity effect (NE), which describes the productivity in mixtures compared to the average of the monocultures and—when positive—indicates overyielding of the mixture. The NE can be partitioned into the complementarity effect (CE; individual species contributing more

to productivity than predicted from monoculture) and the selection effect (SE; covariance of monoculture and mixture productivity, describing the greater probability of more diverse communities including highly productive species which account for the majority of productivity).¹⁹ Distinguishing whether positive biodiversity effects are driven by CEs or SEs is elementary to optimize farm management practices as well as breeding programs.

It is important to note that earlier work has shown that the contribution of CE and SE to the NE can change over time.²⁰ Lately, studies examining temporal dynamics of plant interactions have gained popularity, as they have unraveled important processes that would have gone by unnoticed if not detected through a series of destructive harvests²¹ and have generally contributed to a better understanding of dynamic processes in diverse plant systems.^{22–24} While earlier studies have shown that the amount of intercepted light increases during the growing season,²⁵ to the best of our knowledge, there are no studies that examined temporal changes of light-use associated traits in annual intercropping systems, important information when considering which traits might be the target for—for example—future breeding programs.

In summary, while differences in light use have been detected when crops are grown in mixtures compared to monocultures, there is little knowledge available on how differences in light use between mixtures and monocultures contribute to biodiversity effects and how the partitioning of light among co-occurring crops changes over time. Applying the additive partitioning method in combination with the study of light-use associated plant traits to mixed cropping systems can help to identify mechanisms that lead to yield advantages and can help identify target traits for breeding programs for crop species in mixtures. Therefore, the objectives of this study were (1) to quantify how NE and its two additive components, CE and SE, change over time and (2) how the differences of light-use associated traits in mixtures compared to monocultures contribute to biodiversity effects and how this changes over time in two different intercropping systems. To assess changes in light use over time, we analyzed two traits related to light acquisition (intercepted light and plant height) and two traits related to light conversion (photosynthetic efficiency and capacity) and measured these on a weekly basis. To quantify biodiversity effects, we measured aboveground biomass during weekly destructive harvests and—once available—quantified biodiversity effects based on final seed yields during the later stages of the growing season. As CEs were expected to be particularly strong in mixtures with crops from differing functional groups, we combined oat (*Avena sativa*) with either a legume (lupin, *Lupinus angustifolius*) or a Brassicaceae (camelina, *Camelina sativa*).

2 | METHODS

2.1 | Site description

The site and experimental design are identical to the one used in Engbersen et al.²⁴ The study was carried out at the field site Aprisco

de las Corchuelas, near Torrejón el Rubio, Cáceres, Spain. The site is located at 290 m a.s.l. (39°48'47.9" N 6°00'00.9" W). Total precipitation between February and June 2019 was 77.4 mm, daily average hours of sunshine during the growing season were 10.5 h and daily mean temperatures ranged between 9.6°C and 21.9°C, averaging 16°C. All climatic data are from the national meteorological service (www.aemet.es).

The experimental garden covered 120 m², divided into 480 square plots of 0.25 m² which were arranged in 12 beds of 10 × 1 m, with two rows of 20 plots, resulting in 40 plots per bed. The beds containing the plots were raised by 40 cm above the soil surface. A penetrable fleece was placed on the soil surface, allowing for root growth beyond 40 cm depth. Each bed on top of the fleece was filled by hand with 40 cm homogenized standard, unenriched, local agricultural soil. The soil consisted of 78% sand, 20% silt, 2% clay and contained 0.05% total nitrogen, 0.5% total carbon and 254 mg total P/kg with a mean pH of 6.3.

The experimental garden was irrigated throughout the growing season and all plots received the same amount of irrigation water. The automated irrigation system was configured for a dry threshold of soil moisture at 17% of field capacity and with a target value of 25% of field capacity. When the dry thresholds were reached, irrigation started automatically and irrigated until reaching the target value. Soil moisture was measured in six randomly selected plots at 10 cm below the soil surface with PlantCare soil moisture sensors (PlantCare Ltd.) and the average soil moisture of these six plots defined the soil moisture used for irrigation control.

2.2 | Experimental design

A complete randomized block design with three different crop species and two different diversity levels was used. The crop species were oat (*Avena sativa*, cv. Canyon), lupin (*Lupinus angustifolius*, cv. Boregine) and camelina (*Camelina sativa*, cv. unknown) and the two diversity levels were monocultures and 2-species mixtures. One block consisted of five plots: one plot of monoculture of each of the three species, one plot with an oat–lupin mixture and one plot with an oat–camelina mixture. A monoculture plot consisted of four

identical rows of the respective crop species and a mixture plot consisted of two alternating rows of each crop species, following a speciesA|speciesB|speciesA|speciesB pattern (Figure 1). The sowing densities and sowing depths were: 400 seeds/m², 2 cm for oat, 160 seeds/m², 5 cm for lupin and 592 seeds/m², 0.5 cm for camelina and were based on current cultivation practice.²⁶ A monoculture plot consisted of four rows of 25 seeds of oat, 10 seeds of lupin and 37 seeds of camelina. For mixtures, we followed a substitutive design, where 50% of the seeds for the monocultures was used per species in the mixtures, to sum up to 100% sowing density per plot. Each block was repeated 54 times to allow for 18 destructive harvests with three replicates at each harvest. Sowing was done by hand on 2 and 3 February 2019.

2.3 | Biomass and leaf parameters

After seedling emergence, weekly destructive harvests took place with the first one starting on 21 February 2019 and the last one on 19 June 2019. At each harvest, three individuals per species per plot were randomly marked and harvested as separate individuals. Shoots of the marked individuals were cut at the soil surface and seeds were separated from the shoots once available. Plant height of each marked individual was measured from soil surface to the highest photosynthetically active tissue and plant diameter was measured as the maximum horizontal distance between photosynthetically active tissues of the same plant.

The remaining plants of each species per plot were counted, shoots were harvested and separated into shoots and seeds. All plant samples were dried at 75°C for at least 72 h and weighed. For leaf N analysis, dried leaves of the marked individuals were pooled together, ball-milled to powder either in 1.2 ml tubes with two stainless steel beads in a bead mill (TissueLyserII; Qiagen) for 5 min or with a mixer mill (Mixer Mill MM 200; Retsch) for 30 s. Afterwards, either 100 mg (if available) or 4 mg (if the sample was smaller than 100 mg) of ground leaf material was weighed into tin foil cups or 5 × 9 mm tin capsules and analyzed for N contents. The large samples (100 mg) were analyzed on a LECO CHN628C elemental analyzer (Leco Co.) and the small (4 mg) samples on a PDZ Europa 20-20 isotope ratio

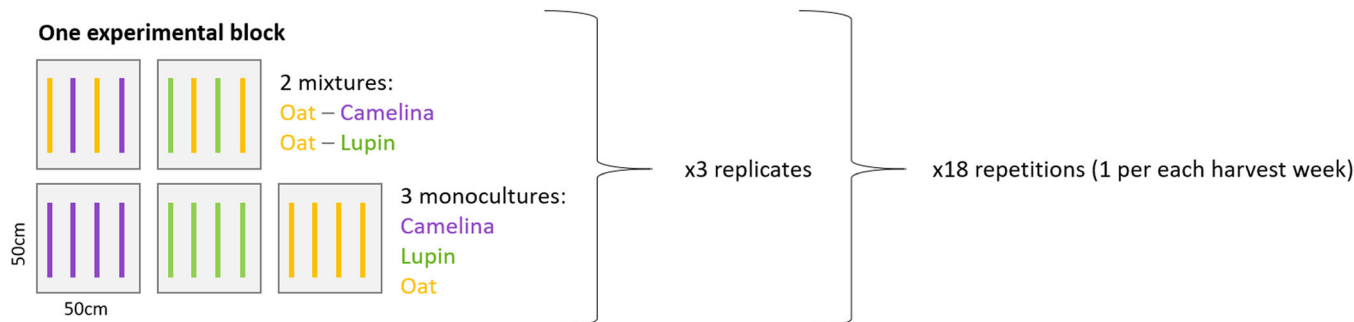


FIGURE 1 Overview of the experimental design. One experimental block consisted of five plots: two mixtures and three monoculture plots. The experimental block was replicated three times and then repeated 18 times to allow for 18 weeks of consecutive, destructive harvests

mass spectrometer linked to a PDZ Europa ANCA-GSL elemental analyzer (Sercon Ltd.).

2.4 | Light measurements

Photosynthetically active radiation (PAR) was measured with an LI-1500 (LI-COR Biosciences GmbH) every week just before the destructive harvest. In each plot, three PAR measurements were taken around noon by placing the sensor on the soil surface in the center of each of the three in-between rows. Light measurements beneath the canopy were put into context through simultaneous PAR measurements of a calibration sensor, which was mounted on a vertical post at 2 m above ground in the middle of the experimental garden. FPAR (%) indicates the fraction of incoming PAR that was absorbed by the crop canopy.

The efficiency of photosystem II photochemistry ($\Phi_{PS_{II}}$) was measured with a fluorometer (MINI-PAM; Walz) equipped with a dark leaf clip (DLC-8). $\Phi_{PS_{II}}$ measures the proportion of light absorbed by chlorophyll associated with photosystem II that is used in photochemistry. As such, it is an indicator of the actual photochemical efficiency.^{27,28} The dark clip was attached to one randomly selected fully developed leaf with no apparent damage. Leaves were dark-adapted for 30 min before applying a saturating actinic light pulse (12,000 $\mu\text{mol photons/m}^2/\text{s}^1$). The light pulse closed all photosystem II reaction centers and allowed determination of the maximum fluorescence of the dark-adapted leaf (F_m) and the leaf's fluorescence shortly before applying the saturation pulse (F). The variable fluorescence F_v was calculated as $F_v = F_m - F$. The maximum efficiency of PS_{II} photochemistry in the dark-adapted state was calculated as $\Phi_{PS_{II}} = F_v/F_m$. Lower values of $\Phi_{PS_{II}}$ indicate a reduced quantum efficiency of photosynthesis, indicating that plants are increasingly stressed.

2.5 | Data analyses

To explain differences in community-level yield between mixtures and monocultures, we quantified the NE and its two additive components, CE and SE, according to Loreau and Hector¹⁹:

$$\Delta\text{yield} = N \times \overline{\Delta RY} \times \overline{M} + N \times \text{cov}(\Delta RY, M), \quad (1)$$

where N is the number of species in the plot. ΔRY is the deviation from the expected relative yield of the species in the mixture in the respective plot, which is calculated as the ratio of observed relative yield of the species in the mixture to the yield of the species in monoculture. M is the yield of the species in monoculture. The first component of the NE equation ($N \times \overline{\Delta RY} \times \overline{M}$) is the CE, while the second component ($N \times \text{cov}(\Delta RY, M)$) is the SE. Yield refers to total aboveground biomass for the harvest weeks (HWs) where no total grain yields were available (i.e., Week 1–14) and to total grain yield when grain yields were available (i.e., Week 17–18). HWs 15–16 were excluded from analyses, as they were not representative for

total biomass anymore due to lupin leaves starting to wilt and fall and not yet representative for total grain yield, as the crop species had not yet produced mature grains.

As the NE and its additive components express the difference in productivity between monocultures and mixtures, we aimed to explain this difference through differences in light-use associated plant traits between mixtures and monocultures. We used a Δ to indicate differences between mixtures and monocultures. Δ trait values were calculated as the difference between community-weighted means of the respective trait value in mixture and monoculture. For example, Δheight was calculated as:

$$\Delta\text{height} = \overline{\text{height}}_{\text{mix}} - \overline{\text{height}}_{\text{mono}}, \quad (2)$$

where $\overline{\text{height}}_{\text{mix}}$ is the average of all three measurements of height per mixture plot and $\overline{\text{height}}_{\text{mono}}$ the average of all three measurements of height of the respective monoculture plot. Weights for community-weighted means were the total biomass of each species. For FPAR, we used mean values instead of community-weighted means.

All statistical analyses were performed in R version 3.6.0.²⁹ We used linear models to explain biodiversity effects (NE, CE and SE) at the community level. We assessed the significance of the fixed effects and interactions using analyses of variance (ANOVA). The fixed effects of the model were the differences between mixtures and monocultures of each light-use associated trait (FPAR, plant height, $\Phi_{PS_{II}}$, leaf N) and the interactions between each of these with HW (as continuous variable) and mixture composition (oat–camelina vs. oat–lupin). The blocking factor was added as an additional fixed effect without interactions. Absolute values of NE, CE and SE were square-root-transformed and the original signs were put back on the transformed values for analysis.¹⁹ We tested for correlation among the light-use associated traits using Pearson's correlation coefficient. If traits were correlated (i.e., Pearson's correlation coefficient > 0.45), we removed one of the two, keeping the one trait which would lead to the best model fit based on the Akaike Information Criterion (AIC).

3 | RESULTS

3.1 | Biodiversity effects

Biodiversity effects were based on total aboveground biomass during the vegetative period (i.e., HWs 1–14) and on total grain yields during the reproductive period (i.e., HWs 17–18). During the vegetative period, the NE and CE were stronger in the oat–lupin compared to the oat–camelina mixture, while the SE did not differ between mixture compositions (Table 1 and Figure 2a–c). All three biodiversity effects increased over time during the vegetative period and for NE and CE, this effect was stronger in the oat–lupin compared to the oat–camelina mixture (interaction $\text{mix} \times \text{HW}$ in Table 1 and Figure 2b). During the reproductive phase, the NE (Figure 2d) and CE (Figure 2e) were significantly higher in the last compared to the second last HW, continuing the same trend of an increase of NE and CE with time. The SE did not

TABLE 1 ANOVA table showing results of linear models testing the effects of block, mixture composition (mix., i.e. oat–lupin vs. oat–camelina), harvest week (HW), the light-use associated traits and all interactions on the net biodiversity effect (NE) and its two additive components, the complementarity (CE) and selection effect (SE)

	Df	NE			CE			SE		
		SS	F value	p Value	SS	F value	p Value	SS	F value	p Value
Block	2	26.47	2.496	0.092	40.02	4.225	0.02	12.46	1.722	0.189
Mix.	1	42.7	8.054	0.006	76.25	16.1	<0.001	1.09	0.302	0.585
HW	1	225.65	42.557	<0.001	116.08	24.51	<0.001	98.08	27.11	<0.001
Δ FPAR	1	96.28	18.159	<0.001	25.62	5.409	0.024	41.96	11.599	0.001
Δ Height	1	492.62	92.908	<0.001	269.77	56.96	<0.001	80.18	22.162	<0.001
Δ ΦPSII	1	0.86	0.163	0.688	2.44	0.516	0.476	4.5	1.243	0.27
Δ Leaf N	1	49.76	9.385	0.003	15.33	3.236	0.078	14.18	3.919	0.053
Δ FPAR × mix.	1	0.63	0.118	0.732	0.26	0.055	0.816	7.68	2.123	0.151
Δ Height × mix.	1	18.56	3.5	0.067	0.23	0.048	0.828	74.6	20.62	<0.001
Δ ΦPSII × mix.	1	0.98	0.186	0.668	0.41	0.086	0.771	3.81	1.053	0.309
Δ Leaf N × mix.	1	30.66	5.782	0.02	5.36	1.132	0.292	108.3	29.921	<0.001
Mix. × HW	1	42.76	8.064	0.006	61.16	12.914	0.001	10.51	2.906	0.094
Δ FPAR × HW	1	6.47	1.22	0.274	8.53	1.801	0.185	0	0.001	0.976
Δ Height × HW	1	0.01	0.002	0.966	0.86	0.181	0.673	0.03	0.009	0.926
Δ ΦPSII × HW	1	2.67	0.504	0.481	2.25	0.475	0.494	0.01	0.004	0.952
Δ Leaf N × HW	1	40.63	7.663	0.008	7.93	1.675	0.201	10.44	2.886	0.095
Δ FPAR × mix. × HW	1	0.78	0.147	0.703	1.3	0.274	0.603	4.57	1.264	0.266
Δ Height × mix × HW	1	17.55	3.309	0.075	54.76	11.562	0.001	63.68	17.602	<0.001
Δ ΦPSII × mix. × HW	1	6.2	1.169	0.285	22.66	4.784	0.033	8.79	2.431	0.125
Δ Leaf N × mix. × HW	1	25.37	4.785	0.033	0.79	0.167	0.684	38.04	10.514	0.002
Residuals	53									

Note: Δ indicates differences of the respective trait between mixtures and monocultures. Biodiversity effects (NE, CE, SE) are based on total aboveground biomass for the vegetative part.

Abbreviations: ANOVA, analysis of variance; F value, variance ratio; FPAR, fraction of incoming photosynthetically active radiation; SS, sum of squares; p value, error probability; ΦPSII, efficiency of photosystem II.

increase with time but was significantly higher in the oat–lupin compared to the oat–camelina mixture (Figure 2F).

3.2 | Light-use-associated traits and biodiversity effects

Collinearity among the light-use associated plant traits occurred between Δ diameter and Δ height (Table S1). Model comparison based on AIC indicated that the model fit improved after removing Δ diameter as explanatory variable from the model.

3.2.1 | Δ FPAR

Increases in Δ FPAR significantly increased with NE, CE and SE (Table 1 and Figures 3a, 4a and 5a) indicating that higher light

interception in mixtures compared to monocultures was positively related to all three biodiversity effects. This effect did not differ significantly between mixture compositions or during the growing season (interactions Δ FPAR × mix and Δ FPAR × HW in Table 1). Although insignificant, the strength of the positive relationship between Δ FPAR and all three biodiversity effects tended to increase with time in the oat–lupin mixture.

3.2.2 | Δ Height

Overall, all three biodiversity effects increased with Δ height (Table 1 and Figures 3b, 4b and 5b). In the oat–lupin mixture, the SE decreased with increasing Δ height during the earlier HWs but the relationship became positive during the later growing season (Figure 5b). In the oat–camelina mixture, the relationship between SE and Δ height changed from positive in the early growing season to

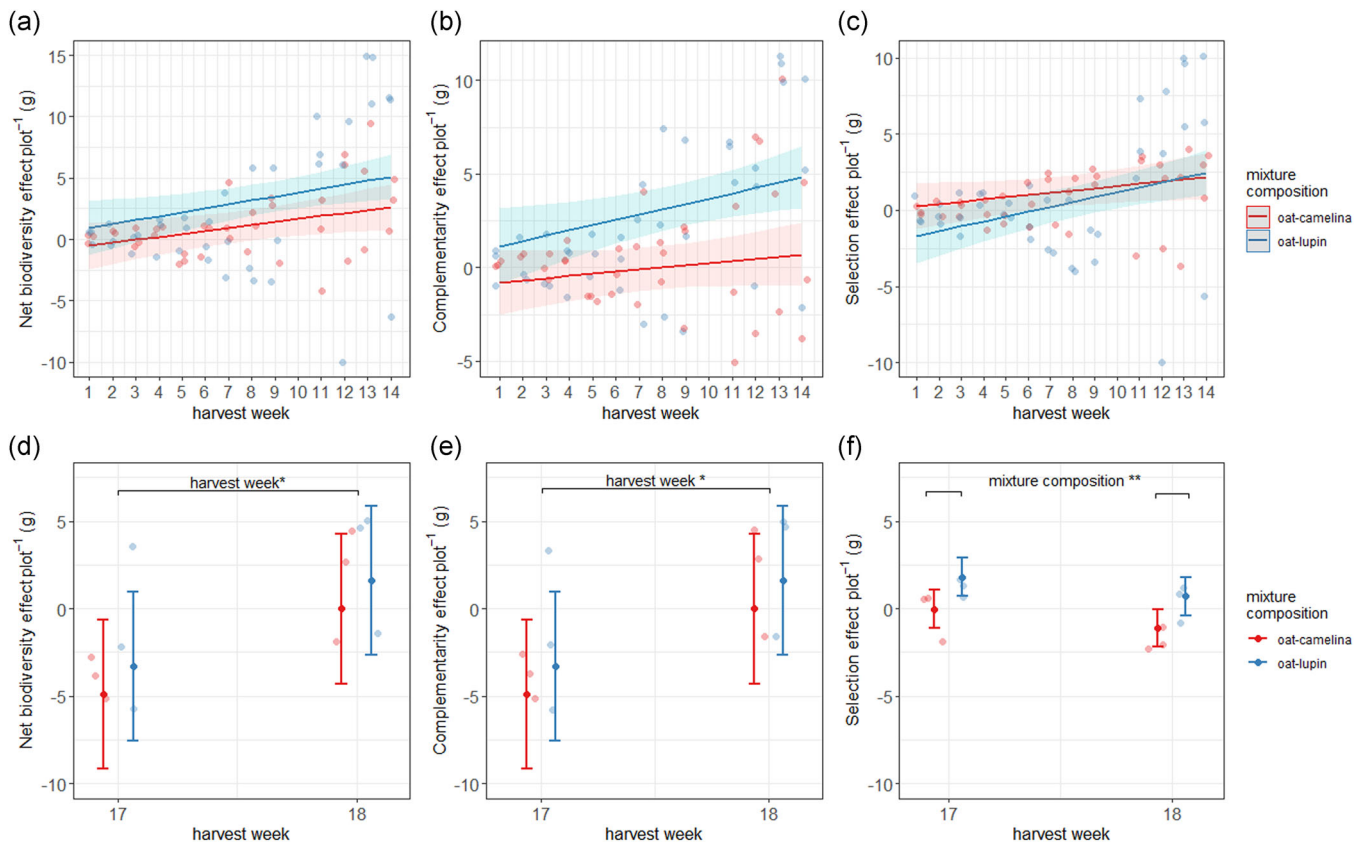


FIGURE 2 The net biodiversity effect (a, d), complementarity effect (b, e) and selection effect (c, f) based on total biomass for the vegetative period (a–c) and based on total grain yields for the reproductive period (d–f) shown for oat–camelina (red) and oat–lupin (blue) mixtures. Lines in (a–c) show the marginal effect associated with the full model presented in Table 1. Data in (d–f) are mean and 95% confidence interval and significance analyses are based on linear models presented in Table S2

negative in the later growing season (Figure 5b). The positive relationship between Δ height and CE increased over time in the oat–camelina mixture but tended to decrease over time in the oat–lupin mixture (Figure 4b).

3.2.3 | Δ Efficiency of PS_{II}

The interaction $\Delta\Phi PS_{II} \times \text{mix} \times \text{HW}$ (Table 1) was significant only for the CE, indicating that in the oat–camelina mixture, the relationship between CE and $\Delta\Phi PS_{II}$ became positive and stronger over time, while in the oat–lupin mixture the relationship remained largely neutral during the entire season (Figure 4c). These results indicated that efficiencies of PS_{II} were comparable between crops in mixture and monoculture in the latter system.

3.2.4 | Δ Leaf N

Δ Leaf N showed an overall positive relationship with NE in the oat–lupin mixture and an overall negative relationship with NE in the oat–camelina mixture (Figure 3d). Over time, the effect became stronger, that is, more positive in the oat–lupin mixture (interaction

Δ leaf N \times mix. \times HW in Table 1 and Figure 3D). Δ Leaf N was negatively correlated to SE in the oat–camelina mixture and positively in the oat–lupin mixture (interaction Δ leaf N \times mix. in Table 1 and Figure 5d). In the oat–lupin mixture, the relationship between Δ leaf N and SE was negative during the early growing season but positive afterwards (interaction Δ leaf N \times mix. \times HW in Table 1 and Figure 5d). No effect of Δ leaf N was observed on CE (Table 1).

4 | DISCUSSION

Understanding the underlying mechanisms of positive biodiversity effects in intercropping systems is essential when developing intercrops as a tool for sustainable agriculture. To address these needs, we investigated how light-use-related traits contribute to the NE via CE or SE and how these relationships change throughout an annual growing season.

We found increasing NE and SE in two different crop mixtures over time during the vegetative period. CEs were found to increase only in the mixture containing a legume. While the NE and CE also increased during the reproductive period, no increase was observed for the SE. This could suggest a discrepancy between the effects of biodiversity on biomass and seed yield.

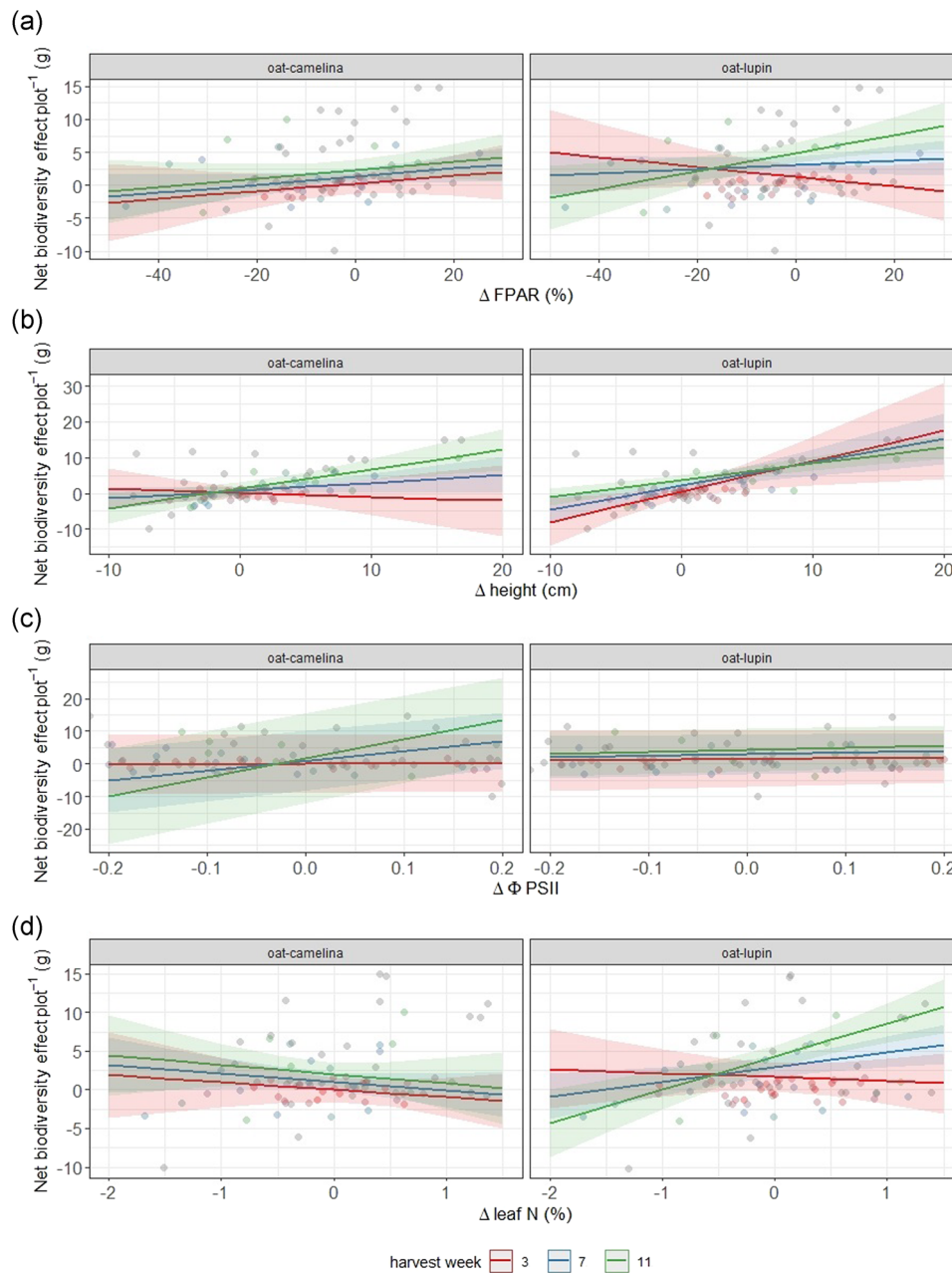


FIGURE 3 Relationships between net biodiversity effect and (a) differences in the fraction of incoming photosynthetically active radiation (FPAR) between mixtures and monocultures (Δ FPAR), (b) differences in height between mixtures and monocultures (Δ height), (c) differences in the efficiency of PSII between mixtures and monocultures (Δ Φ PSII [efficiency of photosystem II]) and (d) differences in leaf N between mixtures and monocultures (Δ leaf N) in the oat–camelina (left panels) and oat–lupin (right panels) mixtures. Colors indicate time points during the growing season with harvest week 3 (red), harvest week 7 (blue) and harvest week 11 (green). Lines show the marginal effect and 95% confidence interval of the full model shown in Table 1

We found that higher light interception in mixtures compared to monocultures contributed positively to the NE through both additive components. Taller plants in mixtures compared to monocultures contributed to the CE in both mixture types while more similar height between mixtures and monocultures contributed to the SE. We also observed differing strategies of light conversion in the two mixtures: While an increased efficiency of PSII contributed to the CE in the oat–camelina mixture, an

increased photosynthetic capacity contributed to the SE in the oat–lupin mixture.

4.1 | Biodiversity effects over time

Increasing biodiversity effects over time are known to occur in long-term studies over time scales ranging from multiple years to

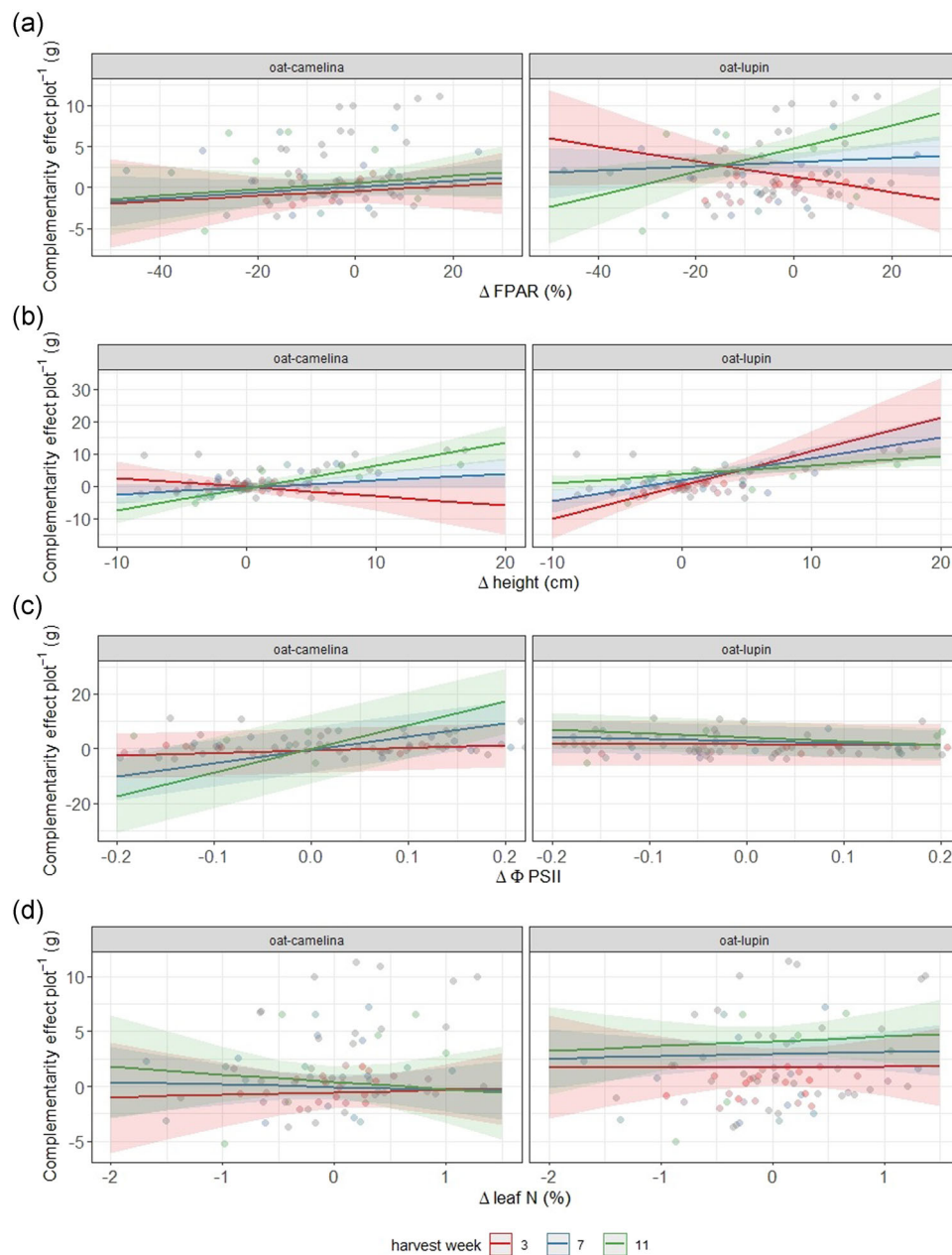


FIGURE 4 Relationships between complementarity effect and (a) differences in the fraction of incoming photosynthetically active radiation (FPAR) between mixtures and monocultures (Δ FPAR), (b) differences in height between mixtures and monocultures (Δ height), (c) differences in the efficiency of PSII between mixtures and monocultures (Δ Φ PSII [efficiency of photosystem II]) and (d) differences in leaf N between mixtures and monocultures (Δ leaf N) in the oat–camelina (left panels) and oat–lupin (right panels) mixtures. Colors indicate time points during the growing season with harvest week 3 (red), harvest week 7 (blue) and harvest week 11 (green). Lines show the marginal effect and 95% confidence interval of the full model shown in Table 1

decades.^{30,31} On a shorter time scale, a recent study has shown that relative contributions of SE and CE to the NE changed over the course of a year in a grassland mixture.³² However, considerably less is known about temporal changes of biodiversity effects over the course of a growing season in annual crop communities. This study found an increase of the NE and its two additive components, the CE and SE, over the lifetime of an annual crop. Naturally, biodiversity effects are expected to increase during the lifespan of annual crops, as interactions between neighboring crops increase as they grow. However, although

this study observed an overall increase of biodiversity effects over time, these relationships differed in the two different mixtures. While the increase of NE and SE with time showed no difference between the mixtures, the CE only increased over time in the oat–lupin mixture but not in the oat–camelina mixture. While the SE increased throughout the vegetative period, the absence of an increase in SE during the reproductive period could be akin to the strong SE observed early during a long-term biodiversity experiment, where the SE decreased with time and eventually even became negative.²⁰

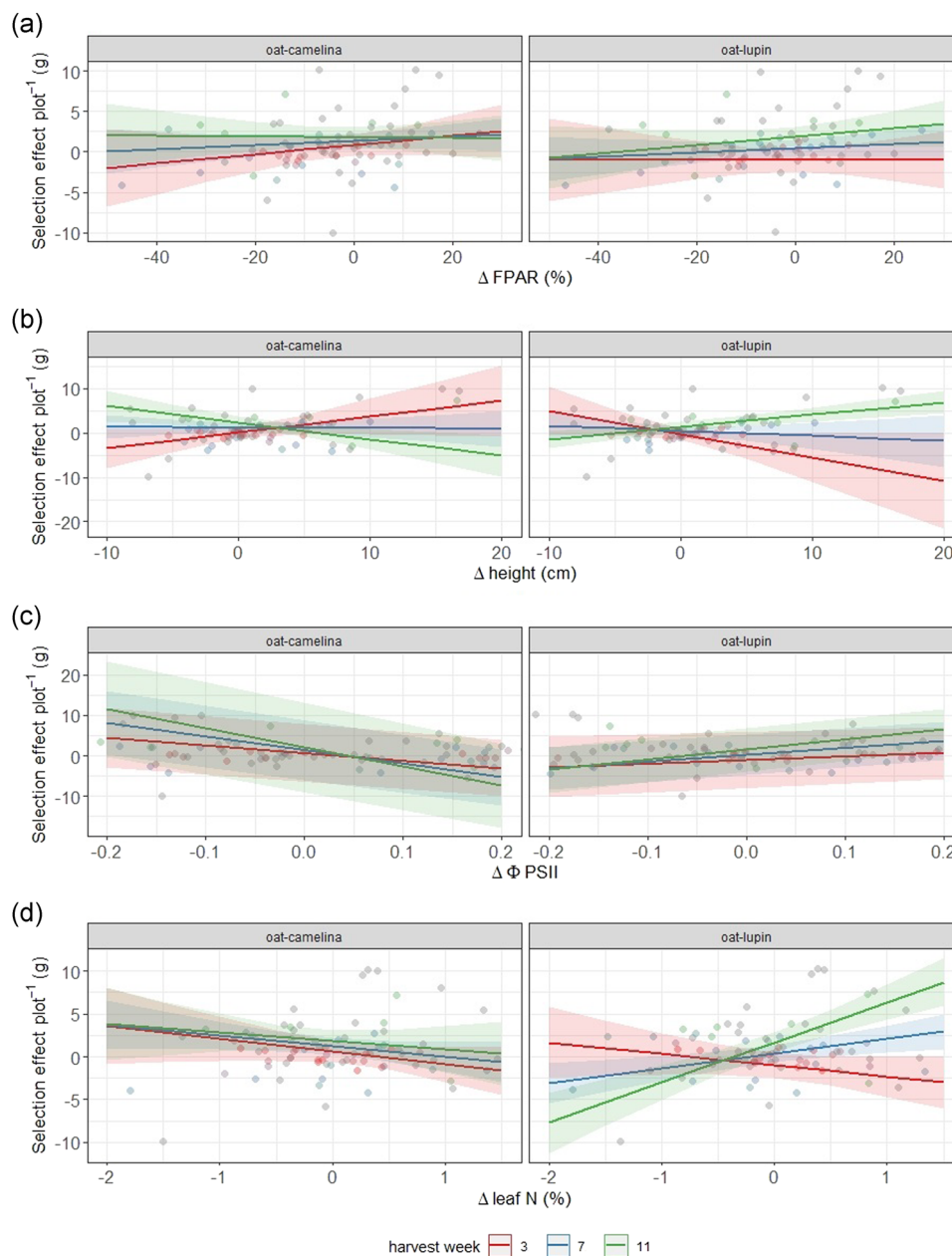


FIGURE 5 Relationships between selection effect and (a) differences in the fraction of incoming photosynthetically active radiation (FPAR) between mixtures and monocultures (Δ FPAR), (b) differences in height between mixtures and monocultures (Δ height), (c) differences in the efficiency of PSII between mixtures and monocultures ($\Delta\Phi$ PSII [efficiency of photosystem II]) and (d) differences in leaf N between mixtures and monocultures (Δ leaf N) in the oat–camelina (left panels) and oat–lupin (right panels) mixtures. Colors indicate time points during the growing season with harvest week 3 (red), harvest week 7 (blue) and harvest week 11 (green). Lines show the marginal effect and 95% confidence interval of the full model shown in Table 1

Alternatively, it could be that the higher biomass of the highly productive species causing most of the SE did not translate into an equally high seed yield. A discrepancy between the effects of diversity on biomass and seed yield has been observed before and is possibly due to currently commercially available crops having a higher harvest index in monocultures than in mixtures.³³ Increasing CEs in the oat–lupin mixture but the absence of a similar increase in the oat–camelina mixture suggests that the presence of the legume potentially contributed strongly to the CE and that cereal–legume mixtures are not

without reason considered a successful combination for intercropping.³⁴ Most complementarity effects in cereal–legume mixtures are attributed to the legume meeting most of its N demand by fixing atmospheric N₂, thus leaving most soil N for the neighboring cereal, which has been observed before for oat–lupin mixtures.²⁴ However, the present study could also show that specifically for the oat–lupin mixture, complementarity in light use due to the differences in canopy architecture between the intercropped species could further contribute to complementarity in this mixture.

4.2 | Biodiversity effects and light-associated traits

All three biodiversity effects increased with increasing FPAR in mixtures compared to monocultures, suggesting that complementary light use is a key process driving intercropping benefits. Higher light interception in mixtures compared to monocultures was probably due to an improved three-dimensional space-filling and greater biomass density in the canopy caused by complementarity in plant architecture between different species.³⁵ These observations are in line with the results from other studies, where the combination of species from different functional groups could add complexity to the canopy structure due to species-specific differences in morphology and increase complementary light use in mixtures.^{35,36} In our study, both mixtures consisted of crop species from different functional groups with quite different canopy structures.

The taller growth of plants grown in mixture compared to in monoculture contributed to the CE due to more complex canopy structures in the mixture. This effect was equally strong throughout the growing season in the oat–lupin mixture but increased over time from a weak to a strong effect in the oat–camelina mixture (Figure 4b). Increased plant height is an indicator for light competition, as growing tall improves the plants' access to light and expresses competitive ability over neighbors.^{37,38} Our observations of increasing plant height in mixtures compared to monocultures are in line with other studies, which found that plants grew taller in maize–soybean mixtures compared to the respective monocultures³⁹ and this could even be linked to yield increases of the crop cultivated in the mixture.⁴⁰ The increasing strength of the relationship between CE and Δ height over time in the oat–camelina mixture was probably due to a slow initial establishment of the camelina in the mixture compared to the camelina in monoculture, which has been observed before in this experiment.²⁴

Our study found that in the oat–camelina mixture, higher efficiency of PS_{II} in mixtures, compared to monocultures, contributed increasingly to the CE over the growing season, but a neutral relationship was observed in the oat–lupin mixture. This could indicate that strategies of shade tolerance were different in the two mixtures, that is, while the oat–camelina mixture increased the photosynthetic efficiency in response to lower light conditions, the oat–lupin mixture may have rather responded by increased photosynthetic capacity. It has been argued that different crops in mixtures have differing strategies for acclimating to their light environments.¹³ Photosynthetic capacity and leaf N are known to be closely linked, as more than 50% of total leaf N is allocated to the photosynthetic machinery (e.g., Rubisco) and other enzymes of the Calvin cycle.⁴¹ This could support the assumption of increased photosynthetic capacity in the oat–lupin mixture, as we observed that higher leaf N in mixtures compared to monocultures contributed to the NE in the oat–lupin mixture. Higher leaf N in oat and lupin, when grown in mixture compared to when grown in monoculture, is in line with earlier observations in this mixed cropping system²⁴ and are due to the lupin meeting its N-demand by symbiotic N_2 -fixation, leaving more soil N for the intercropped oat. However, examining leaf N on a mass basis comes with certain caveats: (1) it does not account for the possibility that nitrogen is likely allocated to different light-harvesting compounds while

total N of the leaf remains the same. For instance, total nitrogen to chlorophyll ratios have been shown to increase in deeper shade among individuals⁴²; (2) leaf N also depends on nutrient availability and competitive ability of the crop in the mixture. We, therefore, highlight the need for more detailed studies investigating the relative contributions of N_2 -fixation and increased photosynthetic capacity and their interdependence, for increasing biodiversity effects in cereal–legume mixtures over time.

5 | CONCLUSION

This study provided evidence that the NE and SE increased over time in both cereal–legume and cereal–nonlegume mixtures, while the CE only increased in the mixture containing a legume. Higher light interception in mixtures compared to monocultures contributed to these positive biodiversity effects in both mixtures and was partly driven by taller plants in mixtures compared to monocultures. While strategies for shade avoidance through taller plants were similar in both mixtures, strategies for shade tolerance differed between the two mixtures. In the oat–lupin mixture, higher photosynthetic capacity in the mixture compared to the monoculture contributed to a positive NE, whereas in the oat–camelina mixture the positive NE was driven by higher photosynthetic efficiency in mixtures compared to monocultures. This study shows that studying the temporal dynamics of biodiversity effects and their relationships to light-use-related traits in intercropping systems can improve our understanding of underlying mechanisms that drive overyielding in annual crop mixtures. This improved understanding of the mechanisms that lead to overyielding is urgently needed to support more frequent use of mixed cropping systems in modern agriculture and the breeding of crop cultivars better suited to intercropped systems. The implementation of more diverse cropping systems has the potential not only to improve yields but can also be a key strategy to increase sustainability in modern agriculture.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

ETHICS STATEMENT

The authors confirm that they have adhered to the ethical policies of the journal.

AUTHOR CONTRIBUTIONS

Nadine Engbersen and Christian Schöb conceived the study with input from Laura Stefan and Rob W. Brooker; Nadine Engbersen and

Laura Stefan collected the data; Nadine Engbersen analyzed samples in the laboratory; Nadine Engbersen assembled and analyzed the data with the help of Christian Schöb; Nadine Engbersen wrote the first draft of the paper. All authors discussed data analyses and results and revised the manuscript.

DATA AVAILABILITY STATEMENT

Data available from Zenodo repository: <https://doi.org/10.5281/zenodo.5040059>.⁴³

ORCID

Nadine Engbersen  <http://orcid.org/0000-0003-4666-9167>

Laura Stefan  <https://orcid.org/0000-0003-0798-9782>

Christian Schöb  <https://orcid.org/0000-0003-4472-2286>

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SUPPORTING INFORMATION

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