DISS. ETH Nr. 27667

Temporal dynamics of above- and belowground resource use in annual intercropping systems

A thesis submitted to attain the degree of

DOCTOR OF SCIENCES of ETH ZURICH

(Dr. sc. ETH Zurich)

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2021

Abstract

Temporal dynamics of resource complementarity in annual intercropping systems is a potentially important factor to optimize crop species mixtures with regards to their resource use, yet they are poorly understood. Intercropping, i.e. the simultaneous cultivation of two or more crop species on the same field, has been demonstrated to be more productive than the respective monocultures. This overyielding is attributed to selection and complementarity effects and little is known about whether the relative contribution of either effect changes over time in annual intercropping systems. Moreover, only few studies have quantified temporal complementarity in above- and belowground resource use in intercropping systems, even though it is a promising mechanism for exploitation in sustainable crop production.

In the first chapter, this thesis examined the contribution of selection and complementarity effects to yield advantages in an extensive mesocosm intercropping experiment. By linking both effects to a set of plant traits indicative for resource use, I could gain a general understanding of which resource use-related processes were contributing to the complementarity or selection effect. I found that selection and complementarity effects contributed equally to overyielding and were linked to distinct plant traits. In the second and third chapter, I explored the temporal dynamics of biodiversity effects, belowground resource use, biomass accumulation and light-use in two different intercropping system (i.e. cereal–legume and cereal–non-leguminous herb). Selection effects increased over time in both mixtures, while complementarity effects increased over time only in the cereal–legume mixture. The two mixtures showed different pathways to overyielding; while yield advantages in the cereal–legume mixtures were related to nitrogen dynamics and increased photosynthetic capacity in the mixture compared to the monoculture, the cereal–non-leguminous herb mixture was characterized by a shift from positive belowground effects to aboveground competition and increased photosynthetic efficiency in the mixture compared to the monoculture but nitrogen played a less important role.

This thesis demonstrated that including temporal dynamics of resource use is essential in intercropping systems to understand the underlying processes that lead to overyielding. By demonstrating that crop mixtures consisting of different functional groups (i.e. legume vs. non-leguminous herb) show different pathways to overyielding, this thesis can contribute to the development of optimized species mixtures for intercropping and aid the advancement of sustainable agricultural practices.

Zusammenfassung

Die zeitliche Dynamik der Ressourcenkomplementarität in einjährigen Mischkulturen ist ein wichtiger Faktor für die Optimierung von Mischkulturen, besonders im Hinblick auf ihre Ressourcennutzung, jedoch sind diese Dynamiken bisher nur unzureichend verstanden. Mischkulturen, d.h. der gleichzeitige Anbau von zwei oder mehr Nutzpflanzen auf einem Feld, ist ertragreicher als ihr Anbau in Monokulturen. Dieser Mehrertrag wird auf Selektions- und Komplementaritätseffekte zurückgeführt. Es ist wenig darüber bekannt, ob sich der relative Beitrag der beiden Effekte im Laufe der Wachstumsperiode in einjährigen Mischkulturen verändert. Zudem haben nur wenige Studien die zeitliche Komplementarität in der ober- und unterirdischen Ressourcennutzung in Mischkulturen quantifiziert, obwohl dies ein vielversprechender Ansatz für eine nachhaltigere Landwirtschaft wäre.

In dieser Arbeit wurde der Beitrag von Selektions- und Komplementaritätseffekten zu Mehr-erträgen in einem extensiven Mischkulturexperiment untersucht. Indem ich beide Effekte mit einigen Pflanzenmerkmalen verknüpfte, welche indikativ für die Ressourcennutzung sind, konnte ich ein allgemeines Verständnis dafür gewinnen, welche ressourcenbezogenen Prozesse zum Komplementaritäts- oder Selektionseffekt beitragen. Ich fand heraus, dass beide Effekte gleichermaßen zu Mehrerträgen beitragen und mit unterschiedlichen Pflanzenmerkmalen verknüpft sind. Des Weiteren untersuchte ich die zeitliche Dynamik der unterirdischen Ressourcennutzung, der Biomasseakkumulation und der Lichtnutzung in zwei verschiedenen Mischkulturen (Getreide–Leguminose und Getreide–nicht-Leguminose). Die beiden Mischungen erreichten Mehrerträge auf unterschiedlichen Wegen; während die Ertragsvorteile in der Getreide–Leguminosen Mischung mit der Stickstoffdynamik zusammenhing, war die Getreide–Nicht-Leguminosen Mischung durch eine Verschiebung von positiven unterirdischen Effekten hin zu oberirdischer Konkurrenz gekennzeichnet, wobei Stickstoff eine weniger wichtige Rolle spielte.

Diese Arbeit zeigt, dass die Einbeziehung der zeitlichen Dynamik der Ressourcennutzung in Mischkulturen wesentlich ist, um die zugrundeliegenden Prozesse zu verstehen die zu Mehr-erträgen führen. Durch den Nachweis, dass Pflanzenmischungen die aus verschiedenen funktionalen Gruppen bestehen (z. B. Leguminosen vs. Nicht–Leguminosen), unterschiedliche Wege zum Mehrertrag zeigen, kann diese Arbeit zur Entwicklung optimierter Artenmischungen für den Mischkulturanbau beitragen und die Weiterentwicklung nachhaltiger landwirtschaftlicher Praktiken unterstützen.

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Acknowledgements

I would first like to thank my supervisor Christian Schöb for his support, for always believing that there could be a story woven out of results that seemed totally random to me at first, for the warm welcome to the field site in Spain and the trust to let us live in your house, for all the advice on how to tame resisting statistical codes into a nice model, for the always very quick feedbacks on all drafts, questions and other issues. Thank you for the freedom you gave me to find my own way through this PhD, I could not have wished for a better supervisor. Secondly, I want to thank Rob Brooker for his insightful feedbacks and all the time he sacrificed to read through a seemingly endless number of drafts. In combination, their advices and inputs helped considerably to make the entire process much less stressful and more enjoyable than it could have been and I am thankful that I could learn from them.

Thank you to Jacob Weiner for taking time to contribute to this work and I am very grateful to have you as an external examiner. Also, thank you to Ruben Kretzschmar for agreeing to chair the defense. Thank you to the Swiss National Science Foundation for funding this work.

I would like to thank the entire staff at the Asociación Aprisco research station in Spain, particularly Elisa, the two Carlos', Sandra and Anja for their company and help during the field experiments. My thanks also go to the staff at the research station in Lindau, Eschikon for letting us use their facilities. Further, I want to thank Björn Studer for his help and endless patience with the IC and ICP. I learnt from Björn to remain optimistic, even when every lab instrument seemed to be conspiring against me. My thanks also go to Annika Ackermann from the Grassland Group and to Britta Jahn-Humphrey and Matti Barthel from the Sustainable Agroecosystems Group for their support in the lab. In general, I want to thank the entire Sustainable Agroecosystems Group for their company, entertaining lunch breaks and good times and the head of the group, Johan Six, for hosting us. It was great to be part of such a diverse group!

A big thank you goes to the basement girls, Laura Stefan and Rafa Conz, for all the random chats, advice on work and bigger life topics and good times and thank you, Rafa, for never getting too annoyed at all the random stuff and tons of samples we stored in the office. I would share an office with you girls again anytime! A very special thank you goes to Laura Stefan for making the fieldwork and general PhDing very enjoyable, by always keeping a good mood or a box of ice cream hidden. I am very happy that we could share so many adventures together, like fighting the bathroom monster in Spain, falling in love with the sheep, discovering Porto or teaching me how

to not make flying flan. I very much valued the privilege of having you as a friend and co-PhD-student to discuss statistics, work, life and knitting patterns and I am looking forward to more skiing (with new skis for you!), hiking and discovering touristy places in the Swiss Alps.

I also want to thank my friends, Annika Berger, Misato Toda, Patrick Kaufmann – for the sometimes too crazy mountain tours -, Michel Phillip, Silvio Bucher and Anine and Mara Lanz – for the less crazy mountain tours - for their support to make sure that I get to spend my weekends in the mountains on more or less enjoyable outings in the Swiss alps. A special thanks goes to Annika, who not only patiently introduced me to the world of mountaineering (I'm still a greenhorn…) but was also a constant pillar from the non-academic-world to remind me that I had actually nothing to complain about. A thank you also to Michel, for your support in my crusade against some statistical problems. I also want to thank Helen Isler and her family for her support and her trust in me, never doubting my progress, and for introducing me to the art of making bread and knitting. A great thank you goes to Misato Toda, for always joining me on whatever crazy hiking idea I came up with, for teaching me how to make Onigiri and for introducing me to the marvelous world of Japanese food. Thank you also to Silvio and Anine, for your good company during the Covid-Lockdown and for never getting tired of fighting against my pink pigs on the grass when playing Carcassonne.

I also want to thank my family for their believe, love, support and patience during the past 3.5 years, particularly my parents for never doubting me and teaching me to be strong and independent, and to my dad for reading my drafts and giving some honest comments. Thank you also to my brother Robin, Saskia and little Elida for the joyful moments and for trying to understand what I was actually doing all the time. And last but not least, thank you, Nicola Haggenmacher for your immense support and caring, for believing in me even when I didn't, for listening to my complaints and being a stable rock in every bigger or smaller crisis.

General introduction

Primary production of modern agricultural systems is largely based on monoculture cropping and requires a heavy use of agrochemicals for fertilization and pest management (Altieri 1999). The high input of chemicals and non-renewable energy into these modern agricultural systems is questioned more and more and it is widely recognized that changes are needed to prevent detrimental effects on ecosystems and public health (Tilman *et al.* 2002). Modern agricultural systems simplify the natural structure over vast areas by reducing the naturally occurring plant diversity to a single arable crop, which needs intensive and costly management practices (Vandermeer 1989). The loss of biodiversity is accompanied by a loss of ecosystem services and intensively managed agricultural areas are therefore often troubled by soil erosion, limited water penetration causing flooding, pests, reduced soil fertility and many other negative aspects. Economic and environmental costs resulting from this degradation can be quite significant, because these degraded ecosystems lack the capacity to sustain themselves and are dependent on external inputs and intensive human interventive management (Altieri 1999).

Promoting on-farm biodiversity is not an unfamiliar practice in agriculture, particularly in developing countries where costly external inputs are limited. Traditional farming practices often rely on a high degree of diversity in the form of mixed cropping or agroforestry to ensure stability of production, reduce insect and disease incidence and enable intensification of production with limited resources (Malézieux *et al.* 2009). Thus, by mimicking natural ecological principles, on-farm biodiversity can improve the effective use of available resources, such as sunlight, soil nutrients or water and naturally limit pests and diseases (Vandermeer 1989). Applying ecological concepts to agricultural production systems with the aim of creating a sustainable agroecosystem with a more efficient production while requiring less chemical input is known as agroecology (Wezel *et al.* 2014). Throughout this thesis, the term agroecology will refer to the definition by Wezel (*et al.* 2014). A wide range of practices is associated with agroecology with the aim to improve the ecological functioning of cropping systems, such as intercropping, crop rotations, reduced tillage, agroforestry and cover cropping (Wezel *et al.* 2014). A particularly promising agroecological concept is intercropping, where two or more crops are cultivated together on the same land at the same time (Willey 1990). In intercropping, crops of different species or cultivars can make use of beneficial plant-plant interactions, e.g. facilitation or resource partitioning, to

sustainably produce a larger yield than when each crop was grown as monoculture. This yield increase, termed overyielding, is a common advantage in intercropping (Vandermeer 1989). However, it is worth mentioning that the outcomes of intercropping systems can be highly variable in magnitude and are often dependent on the context (Li *et al.* 2020).

Mechanisms leading to overyielding

The mechanisms underlying the positive relationship between diversity and productivity remain debated but generally focus around two major types of processes: First, selection effects (SE) encompass the greater probability that more diverse communities include highly productive species or functional groups, which then account for the majority of productivity (Tilman, Lehman & Thomson 1997). For instance, in China, a hotspot of intercropping, a recent meta-analysis has shown that 10% of all yield gains from intercropping were due to selection effects (Li et al. 2020). The second mechanism is the complementarity effect (CE), caused through resource partitioning or facilitation. Resource partitioning is based on the idea that more diverse communities contain species with spatially, temporally or chemically contrasting resource demands, which leads to a more complete exploitation and less competition for available resources in diverse plant communities compared to monocultures (Tilman, Lehman & Thomson 1997; Loreau & Hector 2001). Facilitation involves plants altering their environment in a way that is beneficial to at least one co-occurring species (Brooker et al. 2008). According to the additive partitioning method, the sum of selection and complementarity effects results in the net biodiversity effect (NE). Thus, the NE describes the productivity in mixtures compared to the average productivity of the respective species in monocultures and – when positive – indicates overyielding (Loreau & Hector 2001).

Temporal dynamics of belowground resource use and biomass accumulation

There is abundant evidence for the presence of belowground (Hauggaard-Nielsen, Ambus & Jensen 2001; Li et al. 2018) and aboveground (Ghanbari *et al.* 2010; Yang *et al.* 2017) spatial complementary of resource acquisition in intercropping systems. Nevertheless, it remains debated how much these processes contribute to increased productivity as they are only rarely related to yield or nutrient advantages in the mixed compared to the monoculture communities (Husse et al.

2016; Jesch et al. 2018). In contrast to spatial complementarity, temporal complementarity in resource acquisition has received considerably less attention but has nevertheless been linked to yield advantages, particularly when combining early- and late maturing species in relay intercropping systems (Yu et al. 2015; Zhang et al. 2017; Dong et al. 2018). In addition, earlier work from a long-term biodiversity experiment has shown that the contributions of the complementarity and selection effect to the net biodiversity effect changed over time in semi-natural grasslands (Fargione *et al.* 2007). These observations suggest that the often-overlooked factor of temporal dynamics in resource use in mixed plant communities can potentially reveal important underlying mechanisms that contribute to or are the main driver for overyielding. Particularly short-term temporal dynamics in communities with annual plants have not received much attention. This shortcoming mainly rests on the historical difficulty of measuring temporal dynamics, which requires multiple destructive harvests and is therefore characterized by high labor-intensity and the requirement for large-scale studies (Schofield *et al.* 2018). Even though recent work has found that increasing temporal resolution (i.e. shorter harvesting intervals) improves the accuracy of modelling outputs compared to increasing replication, temporal studies requiring destructive harvests at short intervals remain labor-intensive (Schofield 2020).

The importance of understanding temporal dynamics of resource use in plant systems seems obvious, given that phenological studies have shown that timing is an important ingredient in the functioning of plant communities (Tang *et al.* 2016). Early studies have already observed that temporal differentiation of nutrient uptake among species in plant communities can explain ecosystem functioning in semi-natural grasslands (McKane & Grigal 1990), in natural plant communities (McKane *et al.* 2002) and in forests (Clark & McLachlan 2003). Following up, a study by Trinder *et al.* (2012) used multiple destructive harvests to examine the temporal dynamics of nitrogen uptake and biomass accumulation of *Dactylis glomerata* and *Plantago lanceolata* when grown together compared to when each was grown in isolation. Trinder *et al.* found that in response to interspecific competition (i.e. when grown together) both species shifted their maximum nitrogen uptake and biomass accumulation rate by up to 17 days compared to when cultivated without competition (i.e. when grown in isolation). This observation suggests that the species may have changed their timings to limit direct competition for the same resource. As the study of Trinder *et al.* (2012) was conducted with perennial grassland species and under artificial conditions in a greenhouse, the applicability of the results to annual crop species in agricultural systems had

yet to be shown. Using a wheat/barley–maize relay intercropping system, Zhang *et al.* (2015) could show that intercropping increased the temporal segregation of nitrogen uptake between wheat/barley and maize, indicating that intercropped species aimed to limit direct competition for nitrogen. A similar amplification of the temporal segregation of biomass growth between intercropped species has been observed in an oilseed rape–maize relay intercrop (Dong *et al.* 2018). However, in relay intercrops, crop species are sown at different times, with the second crop often being sown during growth of the first crop. Thus, relay intercrops are characterized by an intentional temporal segregation between the sown species, aiming to elongate the growing season and to shorten the co-growth period to limit interspecific competition (Lithourgidis *et al.* 2011). Studies of temporal dynamics of intercrops sown at the same time are rare, particularly in non-greenhouse settings. One recent pot experiment investigating temporal dynamics of nutrient uptake in two barley cultivars, one early- and one late-maturing, found that peak nitrogen accumulation was advanced for 0.5 days for the early and delayed by 14.5 days for the late cultivar when grown in intra-cultivar competition compared to when grown in isolation (i.e. without competition) (Schofield *et al.* 2019b). However, no temporal shifts were observed in inter-cultivar competition, suggesting that kin recognition played a role in avoiding direct competition. All these studies suggest that temporal dynamics of nutrient uptake and biomass accumulation change when species are grown with neighbors compared to when grown without competition but to the best of our knowledge, these temporal dynamics of nutrient uptake and biomass accumulation have never been examined in annual intercropping systems under field conditions that were not relay intercrops.

Aboveground resource use

Temporal dynamics of nutrient uptake is, however, not the only resource capture that can explain improved ecosystem functioning in mixed communities. Differences in light-use are often associated with higher yields in more diverse communities (Spehn *et al.* 2000). As competition for light is ubiquitous among plants, either through the presence of neighbors or self-shading within the canopy, they have evolved two main strategies as responses to limited availability of photosynthetically active radiation (PAR) (Valladares & Niinemets 2008). On the one hand, shade avoidance refers to a set of traits that enable a plant to reach for light, such as increased plant

height, which is usually achieved by increased investments into stem compared to leaf biomass, stem elongation or reduced branching (Pierik & de Wit 2013). On the other hand, shade tolerance describes the ability of plants to maximize their carbon gain under low light conditions, which can be achieved by either increased photosynthetic capacity or efficiency. Photosynthetic capacity refers to the maximum rate at which a leaf can fix carbon and is therefore tightly linked to leaf nitrogen contents, as the photosynthetic machinery accounts for around half of the leaf N content (Evans 1989). Photosynthetic efficiency refers to the efficiency by which captured light is converted into biomass (Long *et al.* 2006). Species cultivated as intercrops have been observed to show increased leaf nitrogen contents (Franco, King & Volder 2018) and increased efficiency of the photosynthetic apparatus (Gong *et al.* 2019) as a response to lower light conditions when cultivated in an intercropping system. In a maize–soybean relay intercropping system, Gong *et al.* (2015) assessed whether two different varieties of soybean during the seedling stage showed different strategies to deal with shade. The study found that both soybean varieties exhibited similar shade tolerance traits but only one variety showed a clear preference for shade avoidance, resulting in increased plant height of that variety in the intercrop (Gong *et al.* 2015). However, increasing height of a semi-dwarf crop, such as soybean, might not be the best strategy to acquire more light, particularly when intercropped with a tall-growing neighbor such as maize. Hence, Gong *et al.* (2015) also pointed out that the higher growing soybean variety was highly susceptible to lodging, making the shade avoidance strategy a less favorable breeding target in intercrops, particularly when combining understory crops with tall-growing crops. Thus, while favoring traits for shade avoidance is reasonable in some intercropping systems, particularly during the early stages of the growing season, aiming to maximize the efficiency and capacity of photosynthetic processes might be a more promising strategy, particularly during the later stages of a growing season. However, to the best of our knowledge, the interaction of shade avoidance and shade tolerance strategies over an entire annual crop growing season have not yet been examined in intercropping systems and little is known about whether annual crop plants can implement both strategies at different times during the growing season.

Objectives and structure of the thesis

The aim of this thesis is to bring together the temporal dynamics of above- and belowground resource use to improve our understanding of the underlying processes that can lead to overyielding in intercropping systems. By using an ecological approach and applying ecological concepts such as the additive partitioning method (Loreau & Hector 2001) to an agricultural setting, I intend to provide a new perspective on how to unravel underlying causes for successful growth of crop mixtures in agricultural systems. The insights gained from this thesis are intended to help creating more successful species combinations for intercropping systems, based on an in-depth understanding of the underlying temporal processes that ensure maximized ecosystem functioning. Moreover, I intend to show how certain plant traits are related to overyielding via the selection or complementarity effect, aiming to contribute to the slowly growing awareness that cultivars for intercrops require different traits than cultivars for monocultures.

In the first part, described in **Chapter 1**, I used an extensive mesocosm biodiversity experiment with eight different crop species, three different diversity levels and two different climatic environments to assess the contribution of the complementarity (CE) and selection effects (SE) to the net biodiversity effect (NE). Furthermore, I linked the CE and SE to a set of plant traits that were indicative of resource use to gain a general understanding of which resource use-related processes were contributing to the CE or SE. The aim of the first chapter was to derive generalized relationships i) between the NE and its' two additive components, the SE and CE, and ii) between plant traits related to resource use and the biodiversity effects, which would guide more detailed examinations in the following chapters.

Based on insights from the first chapter, in **Chapter 2**, I chose two crop species mixtures (cereal–legume and cereal–non-leguminous herb) to analyze the temporal dynamics of belowground resource use and biomass accumulation. The objective of this chapter was to quantify temporal complementarity of nutrient use and biomass accumulation in annual crop species cultivated with a) the same neighbors (i.e. monoculture), b) different neighbors (i.e. intercrop) and c) no neighbors (i.e. in isolation) at weekly intervals. Besides nutrient and biomass dynamics, I examined the temporal dynamics of further physiological processes that could influence nutrient uptake patterns, such as root exudation and biological N_2-fixation by the legume. My hypotheses were that temporal differentiation within a species (intra-specific) increased or decreased temporal differentiation of i) nutrient uptake or ii) biomass accumulation between different species (inter-

specific) in an intercrop. I further hypothesized that iii) root exudation differed temporally or quantitatively between crop species cultivated with the same neighbors, different neighbors or no neighbors and that iv) this could be linked to increased phosphorous uptake of the exuding or neighboring species. Finally, I hypothesized that v) biological N_2-fixation of the legume would increase when intercropped with the cereal.

In **Chapter 3**, I used the same experimental setup and crop mixtures as in chapter two and quantified how the biodiversity effects (NE, CE and SE) changed over time in the two mixtures. Furthermore, I quantified how differences in traits associated with either shade avoidance or shade tolerance between mixtures and monocultures contributed to the biodiversity effects and how these relationships changed throughout the growing season. My hypotheses were that i) the biodiversity effects would increase over time and that ii) the CE would contribute more strongly to the NE than the SE. I further hypothesized that iii) the SE would be mainly related to increased plant height in mixtures compared to monocultures, indicating competitiveness in light-use acquisition of a highly productive species, and that iv) the CE would be related to a wider range of traits associated to shade avoidance and shade tolerance.

Chapter 1

Using plant traits to understand the contribution of biodiversity effects to annual crop community productivity

Abstract

Increasing biodiversity generally enhances productivity through selection and complementarity effects not only in natural but also in agricultural systems. However, the quest to explain why diverse cropping systems are more productive than monocultures remains a central goal in agricultural science. In a mesocosm experiment, we constructed monocultures, 2- and 4-species mixtures from eight crop species with and without fertilizer and both in temperate Switzerland and dry, Mediterranean Spain. We measured environmental factors and plant traits and related these in structural equation models to selection and complementarity effects to explain seed yield differences between monocultures and mixtures. Increased crop diversity increased seed yield in Switzerland but not in Spain. This positive biodiversity effect was driven to almost the same extent by selection and complementarity effects, which increased with plant height and SLA, respectively. Also, ecological processes driving seed yield increases from monocultures to mixtures differ from those responsible for seed yield increases through the diversification of mixtures from 2 to 4 species. While selection effects were mainly driven by one species, complementarity effects were linked to larger leaf area per unit leaf weight. Seed yield increases due to mixture diversification were driven only by complementarity effects and were not mediated through the measured traits, suggesting that ecological processes beyond those measured in this study were responsible for positive diversity effects on yield beyond 2-species mixtures. By understanding drivers of positive biodiversity–productivity relationships, we can improve our ability to predict species combinations that enhance ecosystem functioning and can promote sustainable agricultural production.

Key words: agroecology, biodiversity effects, biodiversity–productivity, complementarity effects, selection effects, crop mixtures, intercropping, plant traits

1.1. Introduction

Plant primary productivity increases with higher species diversity in semi-natural grasslands (e.g. Tilman *et al.* (2001); Cardinale *et al.* (2006)). While the majority of research in this area has been done in perennial systems (Tilman *et al.* 2001; Huang *et al.* 2018), recent studies have demonstrated similar effects in annual systems (Li *et al.* 2014; Brooker *et al.* 2015; Stomph *et al.* 2020). Intercropping of annual crops, where at least two crop species are grown in close proximity at the same time, is therefore a promising application of agroecological concepts. By making use of beneficial above- and belowground species interactions, intercropping can lead to overyielding, which is the increased yield in a mixture compared with the average of the monocultures (Vandermeer 1989).

Two main mechanisms have been proposed to explain positive biodiversity–productivity relationships. First, sampling or selection effects (SE) encompass the greater probability that more diverse communities include highly productive species or functional groups, which then account for the majority of productivity (Tilman, Lehman & Thomson 1997). Enhanced ecosystem functioning in diverse agroecosystems can be driven by SE (i.e. communities with more species are more likely to host a high-performing species). For instance, in China, a hotspot of intercropping, a recent meta-analysis has shown that 10% of all yield gain of intercropping compared to sole cropping was due to selection effects (Li *et al.* 2020).

The second mechanism is the complementarity effect (CE), caused through resource partitioning or facilitation. Resource partitioning involves more diverse communities containing species with contrasting demands on resources, which leads to a more complete exploitation of available resources in diverse plant communities compared with monocultures and hence increased productivity (Tilman, Lehman & Thomson 1997; Loreau & Hector 2001). The partitioning of resources can occur across spatial, temporal or chemical gradients. Belowground, different rooting depths allow plants to take up water or nutrients from different soil layers, thus limiting competition. While this has been observed for water uptake (Miyazawa *et al.* 2009), the evidence of resource partitioning for soil nutrients as a driver of biodiversity effects is less clear (Von Felten *et al.* 2012; Jesch *et al.* 2018). Aboveground, diverse communities can harbor more diverse canopy growth forms allowing for a more complete use of photosynthetically active radiation (PAR) (Spehn *et al.* 2002; Fridley 2003), leading in some cases to yield advantages in mixtures compared with monocultures (Bedoussac & Justes 2010).

Facilitation involves plants altering their environment in a way that is beneficial to at least one co-occurring species (Brooker *et al.* 2008). and can happen via either the enrichment of resource pools by one plant and also benefitting neighboring plants or the mediation of physical or biological stress. The facilitative benefits of legumes increasing soil N for their neighbors by using atmospheric N_2 as their N source are well known, especially for cereals intercropped with legumes (Spehn *et al.* 2002; Temperton *et al.* 2007). Other below-ground facilitative mechanisms shown to occur in intercrops include enrichment of resource pools by hydraulic lift, which not only facilitates water uptake (Sekiya & Yano 2004), but can also enhance nutrient mobilization and lead to improved nutrient status of the intercrop (Sun *et al.* 2013). Other evidence of facilitation mediating physical stress in crop systems comes from studies of barley variety mixtures, where a denser canopy structure, shading of the soil surface, and thus reduced evaporation were observed to decrease the soil temperature (Cooper *et al.* 1987). Also, plant species can alleviate the microclimate for their neighbors by mediating wind, heat or photoinhibition (Wright *et al.* 2017). Different types of biotic facilitation are present in diverse systems but are not addressed in this study (Wright *et al.* 2017).

However, despite these examples of different resource partitioning and facilitation mechanisms occurring in crop systems, knowledge about the precise mechanisms that lead to overyielding in crop mixtures, and how environmental conditions can alter these mechanisms, still remains incomplete (Duchene, Vian & Celette 2017). While there is abundant evidence for the presence of complementarity effects in diverse agricultural systems, the presence of these processes alone does not guarantee overyielding (Barry *et al.* 2019).

Here, we applied ecological methods to a setting with crop species of agricultural importance by assessing to what extent CE and SE drive yield gains in crop mixtures compared to crop monocultures and how these effects are related to differences in plant functional traits and environmental factors between mixtures and monocultures. We used the additive partitioning approach by Loreau and Hector (2001) to quantify CE and SE. By linking frequently-used plant traits to yield gains in mixtures, we can improve our ability to predict optimal species combinations which can help to promote sustainable agricultural production through intercropping. We used four plant traits indicative of resource use: leaf dry matter content (LDMC), specific leaf area (SLA), carbon to nitrogen ratio (C:N ratio) and plant height and two environmental factors: Photosynthetic active radiation (PAR) and volumetric soil water content (VWC). We used a

structural equation model (SEM) to assess whether overyielding is driven by selection or complementarity effects or by a combination of both. Furthermore, we used this hierarchical model to understand the context–dependence of the CE and SE and how they are linked to plant functional traits.

1.2. Materials and Methods

1.2.1. Site description

The study was carried out in two outdoor experimental gardens in Zürich, Switzerland and Torrejón el Rubio, Cáceres, Spain. The Swiss site was located at an altitude of 508 m a.s.l. (47°23'45.3" N 8°33'03.6" E), the Spanish site at 290 m a.s.l. (39°48'47.9" N 6°00'00.9" W). Switzerland is characterized by a temperate climate, Spain by a dry, Mediterranean climate. Main climatic differences during the growing season between the two sites were precipitation (Switzerland: 587 mm, Spain: 218 mm), daily average sunshine hours (Switzerland: 5.8h, Spain: 9.6h), while mean temperatures were comparable (Average of daily mean, minimum and maximum temperature in Switzerland: 15.8 °C, 10.9 °C, 21.1 °C; in Spain: 15.5 °C, 9.7 °C, 21.4 °C) All climatic data are from the respective national meteorological services and are average values over the growing season (www.meteoschweiz.admin.ch, www.datosclima.es).

The experimental garden at each location covered 69.5 m^2, divided into 278 square plots of 0.25 m^2 and 40 cm depth. Plots were open at the bottom to allow root growth beyond 40 cm. Inside a bed, plots were separated from each other by metal frames. In Switzerland, the plots were arranged in 10 beds of 7×1 m, with two rows of 14 adjacent plots, resulting in 28 plots per bed. In Spain, the plots were arranged in 14 beds of 10×1 m, with two rows of 20 adjacent plots, resulting in 40 plots per bed. Two corner plots in each location were left empty. The plots were filled with local, unenriched agricultural soil. Soil structure and composition therefore differed between the sites. In Switzerland, soil was composed of 45% sand, 45% silt, 10% clay and contained 0.19% nitrogen, 3.39% carbon, and 333 mg total P/kg with a mean pH of 7.25. Spanish soils consisted of 78% sand, 20% silt, 2% clay and contained 0.05% nitrogen, 0.5% carbon, 254 mg total P/kg with a mean pH of 6.3.

The experimental gardens were irrigated throughout the growing season with the aim of maintaining the differences in precipitation between the two sites but assuring survival of the crops during drought periods. In Switzerland, the dry threshold was set to 50% of field capacity, with a

target of 90% of field capacity, while in Spain the thresholds were 17% and 25% of field capacity, respectively. Automated irrigation was configured such that irrigation would start if the dry threshold was reached and irrigate until the target threshold was reached.

At each site, half of the beds were chosen randomly to be fertilized with N-P-K (1-1.7-1) while the other half served as unfertilized controls. Fertilizer was applied three times: 50 kg/ha just before sowing, another 50 kg/ha when wheat was tillering and 20 kg/ha when wheat was flowering.

1.2.2. Crop species and cultivars

At each site, experimental communities were constructed with eight annual crop species of agricultural interest. The crop species belonged to four different phylogenetic groups and can be seen in Table A1 (Appendix 1: Table A1). The four phylogenetic groups were based on their phylogenetic distances: Cereals diverged from the other groups 160 million years ago (mya); superasterid herbs diverged from superrosid herbs including legumes 117 mya and finally, legumes diverged from the other superrosid herbs 106 mya (*TimeTree*). Phylogenetic distance was chosen as a criterion for functional similarity as it is often positively correlated with functional diversity and acts as a proxy to assess the impacts of species diversity on ecosystem functions (Mouquet *et al.* 2015). At both sites, we grew commercial cultivars typically used for organic farming in Switzerland (Appendix 1: Table A1). While these were bred for a Swiss climate, their cultivation in Spain demonstrated the ability of these cultivars to adapt to a climate change-type scenario, with conditions considerably drier than in Switzerland.

1.2.3. Experimental crop communities

Experimental crop communities at each site consisted of eight different monocultures, 24 different 2-species mixtures consisting of two different phylogenetic groups and 16 different 4-species mixtures consisting of four different phylogenetic groups. Every combination of 2-species mixture with two species from different phylogenetic groups and every possible 4-species mixture with species from four different phylogenetic groups were planted. Each experimental community was replicated two times. The entire setup was repeated two times to allow for fertilizer treatment, resulting in 192 plots per site. Plots were randomized within each country and fertilizer treatment. Each monoculture and mixture community consisted of one, two or four crop species planted in four rows, following a speciesA|speciesB|speciesA|speciesB row pattern for the two-species

mixture and a speciesA|speciesB|speciesC|speciesD row pattern for the four-species mixture. The row order of the species was randomized. Sowing densities differed among phylogenetic groups and were based on current cultivation practice (Appendix 1: Table A1). Sowing was done by hand in early February 2018 in Spain and early April 2018 in Switzerland. Weed plants were removed in May 2018 in Spain (85 days after sowing) and in June 2018 in Switzerland (70 days after sowing). We are aware that the use of 0.25 m^2 does not reflect a realistic agricultural setting. However, using small plots is common in these kind of experiments (Jochum *et al.* 2020) and it allowed us to accommodate a large number of combinations and treatments that would otherwise not have been possible. Furthermore, covering only 70 m^2 of experimental field garden and using the same homogenized soil in all plots at each location allowed us to keep environmental conditions and climatic differences constant throughout one location.

1.2.4. Data collection

Leaf traits were measured at the time of flowering (Spain: May 2018, 94 days after sowing; Switzerland: June 2018, 65 days after sowing). Three individuals per crop species per plot were randomly marked and their height measured with a ruler from the soil surface to the highest green tissue. Trait measurements were done on these three marked individuals. Of each marked individual, 1 to 10 healthy leaves were sampled and immediately wrapped in moist cotton and stored overnight at room temperature in open plastic bags. For the subsequent leaf trait measurements (specific leaf area [SLA] and leaf dry matter content [LDMC]) we followed standard protocols (Cornelissen *et al.* 2003).

At the time of harvest (duration of growing season: Appendix 1: Table A2) all crops in each plot were harvested. The three marked individuals used for the trait measurements were collected separately, while all remaining plants per crop species per plot were pooled together. Plant shoots were cut at the soil surface and biomass and seeds were separated. The total number of individuals per crop species per plot was recorded. Fruits were air-dried. Afterwards, seeds were separated from chaff with a threshing machine (in Switzerland: Allesdrescher K35, Baumann Saatzuchtbedarf, Germany; in Spain: Hege 16, Wintersteiger, Austria). Vegetative biomass was oven-dried at 80 °C until constant weight.

Interception of PAR by the plant canopy was measured weekly with a LI-1500 (LI-COR Biosciences GmbH, Germany). 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 percentage of PAR that was intercepted by the crop canopy. VWC in the upper 6 cm of soil was measured weekly with a ML3 ThetaProbe Soil Moisture Sensor (Delta-T, Cambridge). We used a standard calibration for mineral soil. The measurements were taken in the center of each of the three in-between rows per plot. For further data analysis, we used FPAR and VWC values from the week of leaf trait measurements (Spain: 92 days after sowing; Switzerland: 62 days after sowing).

1.2.5. Plant N analyses

For chemical analyses of the plant tissue we pooled the three dried leaf samples of the marked individuals per plot and per crop species. Leaf samples were ground for 20 minutes in 1.2 ml tubes with two stainless steel beads in a bead mill (TissueLyserII, Qiagen). Afterwards, either 100 mg (if available) or 4 mg (if the sample was too small) of ground leaf material were weighed into tin foil cups or 5×9 mm tin capsules and analyzed for C and N contents. The 400 large samples were analyzed on a LECO CHN628C elemental analyzer (Leco Co., St. Joseph, USA) and the 505 small samples on a PDZ Europa 20-20 isotope ratio mass spectrometer linked to a PDZ Europa ANCA-GSL elemental analyzer (Sercon Ltd., Cheshire, UK), respectively. Eight samples were cross-referenced on both analytical devices (Appendix 1: Fig. A1) and measured values from LECO were corrected to account for the differences between the devices (correction factors are 1.0957 for N and 1.026 for C, respectively).

1.2.6. Data analysis

Prior to data analysis, we eliminated plots with incomplete data. Due to birds foraging on seeds, a substantial number of plots were discarded. A total of 314 plots remained, 160 in Switzerland and 154 in Spain.

To explain differences in community-level yield between mixtures and monocultures, we calculated Δyield (commonly referred to as the net biodiversity effect) as the difference between the summed community-level yields of all species in a mixture plot and the average of the mean community-level yields of all monocultures corresponding to the species in the mixture plot. The minuend and subtrahend were square root transformed to meet the assumptions of normal

distribution. Thus, Δyield compares the observed yield in the mixture with the expected yields in a mixture based on their yields in a monoculture.

We quantified the net biodiversity effect (Δyield) and its two additive components, the CE and SE according to Loreau and Hector (Loreau & Hector 2001).

$$\Delta yield = N \cdot \overline{\Delta RY} \cdot \overline{M} + N \cdot cov(\Delta RY, M) \qquad (1.1)$$

Where N is the number of species in the plot. ΔRY is the deviation from expected relative yield of the species in mixture in the respective plot, which is calculated as the ratio of observed relative yield of the species in mixture to the yield of the species in monoculture. M is the yield of the species in monoculture. The first component of the net biodiversity effect equation ($N \cdot \overline{\Delta RY} \cdot \overline{M}$) is the CE, while the second component ($N \cdot cov(\Delta RY, M)$) is the SE.

All statistical analyses were performed in R version 3.6.0. (R Core Team 2019). We used general linear mixed-effects models using restricted maximum likelihood estimation to explain yield at the community-level. We assessed the significance of the fixed effects using type-I ANOVA and the Satterthwaite approximation of denominator degrees of freedom (*lme4* (Bates *et al.* 2015) and *lmerTest* (Kuznetsova, Brockhoff & Christensen 2017) packages). Yield always refers to seed yield and was log transformed. The fixed effects of the model were country (Switzerland versus Spain), fertilizer (fertilized versus unfertilized), species number (2 versus 4) nested in diversity (monocultures vs mixtures), and interactions among the fixed effects (except between the nested terms). Random terms were species composition and the interactions between garden bed and all fixed effects (fertilizer, legume presence, species number and diversity). To test for effects of diversity within each country, we conducted post-hoc contrasts using the *emmeans* package with Tukey adjustment for multiple comparisons (Lenth 2016).

We used linear mixed-effects models to analyze treatment effects on environmental factors (FPAR, VWC), community-weighted means of plant traits (SLA, LDMC, height, leaf N, C:N ratio) and the two components of the net biodiversity effect (SE, CE) with country, fertilizer, species number nested in diversity and interactions among these as fixed effects. Random terms were species composition and the interactions between garden bed and all fixed effects (fertilizer, legume presence, species number and diversity). Response variables were log transformed, except for SE and CE. Total aboveground biomass of each species was used as weights for community-weighted means of trait values (Roscher *et al.* 2012). These models were fitted with the *asreml* function in the *asreml* package in R and results were extracted using the *test.asreml* function in

the *pascal* package (Niklaus 2019). We used binomial distributions for % data (FPAR, VWC, leaf N), Gamma distributions for ratios (SLA, LDMC, C:N ratio) and Poisson distribution for count data (plant height).

We constructed a piecewise SEM from the *piecewiseSEM* package (Lefcheck & Freckleton 2016) to analyze significant pathways of interactions between the parameters in our model. We started with an *a priori* model with the aim of analyzing significant pathways between environmental factors, differences in plant traits between mixtures and monocultures and selection and complementarity effects (Appendix 1: Fig. A2). We then used the *dSep* function for tests of directed separation, which allowed us to re-include significant direct or indirect paths that were missing in our *a priori* model. The inclusion of each missing pathway was evaluated with the Akaike Information Criterion (AIC), to estimate the robustness of the current model compared to other models of the same dataset. Whenever the tested pathways were significant ($p < 0.05$) and the new model generated a lower AIC score, they were included. If traits strongly correlated, we removed one of the two, keeping the one trait which would lead to the best model fit. When the final model was reached (lowest AIC score with the most variables included) we evaluated the goodness-of-fit of the model by using chi-square statistics (Shipley 2009; Lefcheck & Freckleton 2016). If the χ^2 was statistically non-significant ($p > 0.05$) it indicated a good fit of the model to the data. For each variable, we report the conditional coefficient of determination (R^2_c), which represents the variance explained by fixed and random effects.

Since the additive components of the net biodiversity effect, the CE and SE, express the difference in productivity between monocultures and mixtures, we aimed to explain this difference through differences in environmental and plant characteristics between monocultures and mixtures. We used a Δ to indicate differences between monocultures and mixtures. We calculated ΔVWC and ΔFPAR according to equation 1.2.

$$\Delta VWC = \overline{VWC}_{mix} - \overline{VWC}_{mono} \tag{1.2}$$

Where $\overline{VWC}_{mix}$ is the average of all three measurements of VWC per mixture plot and $\overline{VWC}_{mono}$ the average of all three measurements of VWC of the respective monoculture plots. The same was calculated for ΔFPAR. To scale up plant trait measurements to the community level, community-weighted means in mixtures and monocultures were used to calculate ΔSLA, ΔLDMC, ΔC:N ratio and Δheight:

$$\Delta SLA = CWM.SLA_{mix} - CWM.SLA_{mono} \tag{1.3}$$

Where CWM.SLA$_{mix}$ is the community-weighted mean of SLA of all species in a mixture plot and CWM.SLA$_{mono}$ the community-weighted mean of SLA in monoculture of all the species in the respective plot. Aboveground biomass of each species was used as weights. ΔLDMC, ΔC:N ratio and Δheight were calculated likewise according to equation 1.3.

1.3. Results

1.3.1. Response of community-level yield to treatments

Community-level yield was significantly affected by country, with 88% higher yields in Switzerland compared with Spain (Fig. 1.1**a**, Appendix 1: Table A3). Tukey post hoc tests revealed that productivity of mixtures was enhanced, particularly in Switzerland (Appendix 1: Table A4), where 4-species mixtures yielded 30% more than 2-species mixtures and 93% more than monocultures. Also, 2-species mixtures in Switzerland yielded 48% more than monocultures. The interaction of country × legume indicated that mixtures with legumes yielded more than mixtures without legumes in Spain while in Switzerland, mixtures with legumes yielded less than mixtures without legumes (Fig. 1.1**b**, Appendix 1: Table A4).

1.3.2. Environmental factors, plant traits and biodiversity effect components

Environmental factors did not differ significantly between diversity treatments (Fig. 1.2, Appendix 1: Table A5, A6). Limited water input resulted in a significantly lower volumetric soil water content (VWC) in Spain. However, VWC did not vary significantly in response to fertilizer or diversity treatments (Fig. 1.2**a,** Appendix 1: Table A6). Both plant height and FPAR did not respond to fertilizer treatment but were significantly higher in Switzerland than in Spain, indicating that canopy closure was more complete and that vegetative growth was generally stronger in Switzerland than in Spain (Fig. 1.2**b**, **e**). Plant height was significantly higher in mixtures compared with monocultures and in 4- compared with 2-species mixtures (Fig. 1.2e, Appendix 1: Fig. A4). SLA of crops was significantly higher in Switzerland and LDMC showed an opposite behavior, with higher values in Spain than in Switzerland (Fig. 1.2**c, d**, Appendix 1: Fig. A4). Neither LDMC nor SLA responded significantly to fertilizer or diversity treatments. Leaf N did not differ between treatments (Appendix 1: Table A6). C:N ratio was significantly higher in

Switzerland than in Spain (Fig. 1.2**g**, Appendix 1: Table A5, A6). CE was stronger in Switzerland than in Spain and stronger in 4-species than in 2-species mixtures in Switzerland (Fig. 1.2**h**, Appendix 1: Fig. A4). SE showed no response to any treatment factor (Fig. 1.2**i**).
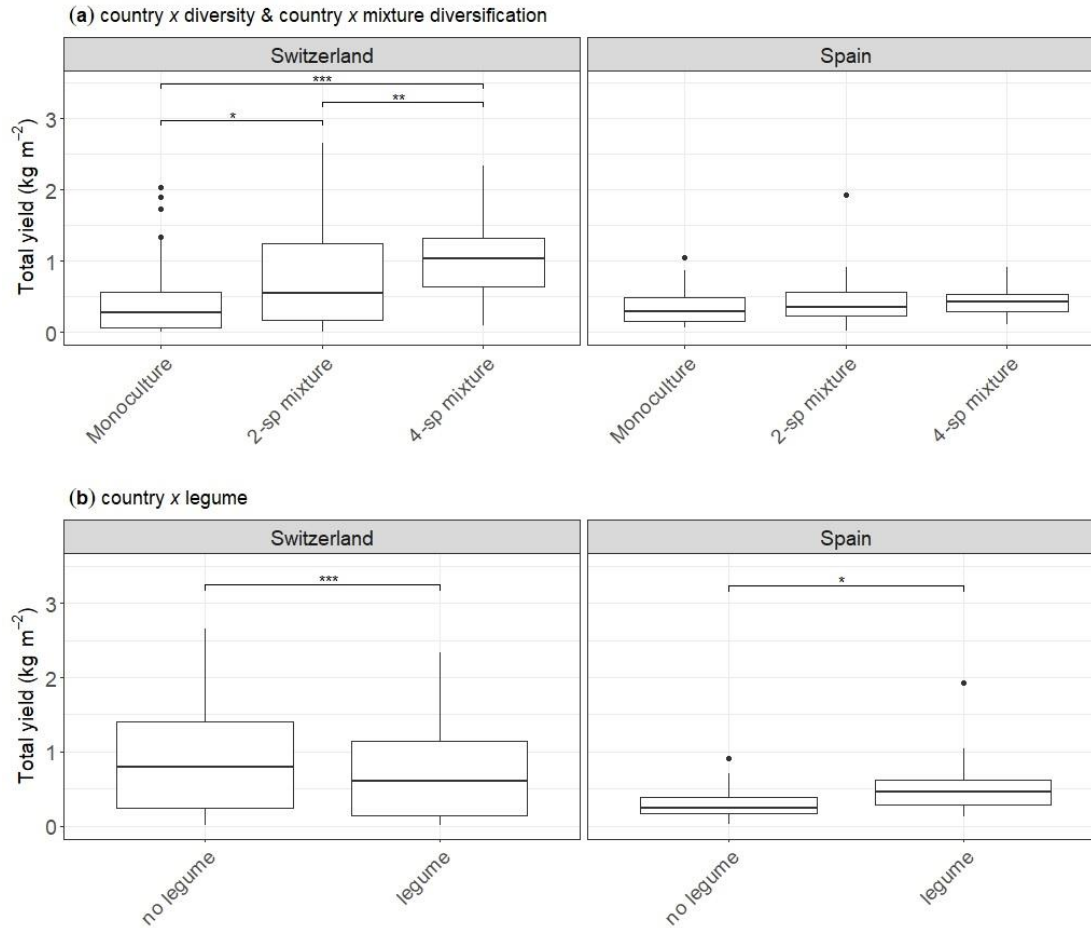
*Figure 1.1: Community-level yields in kg dry weight per m² visualizing the significant results from appendix 1: table A3. Differences in community-level yield between countries (**a**), diversity levels (**a**), mixture diversification (2- vs. 4-species mixtures) (**a**), between the two-way interactions country × legume (**b**), country × diversity (**a**) and country × mixture diversification (**a**) and country × legume (**b**). Brackets indicate significant differences between treatments and labels above brackets indicate which treatment was significant at α = 0.05 (* P < 0.05, ** P < 0.01, *** P < 0.001). n = 314.*
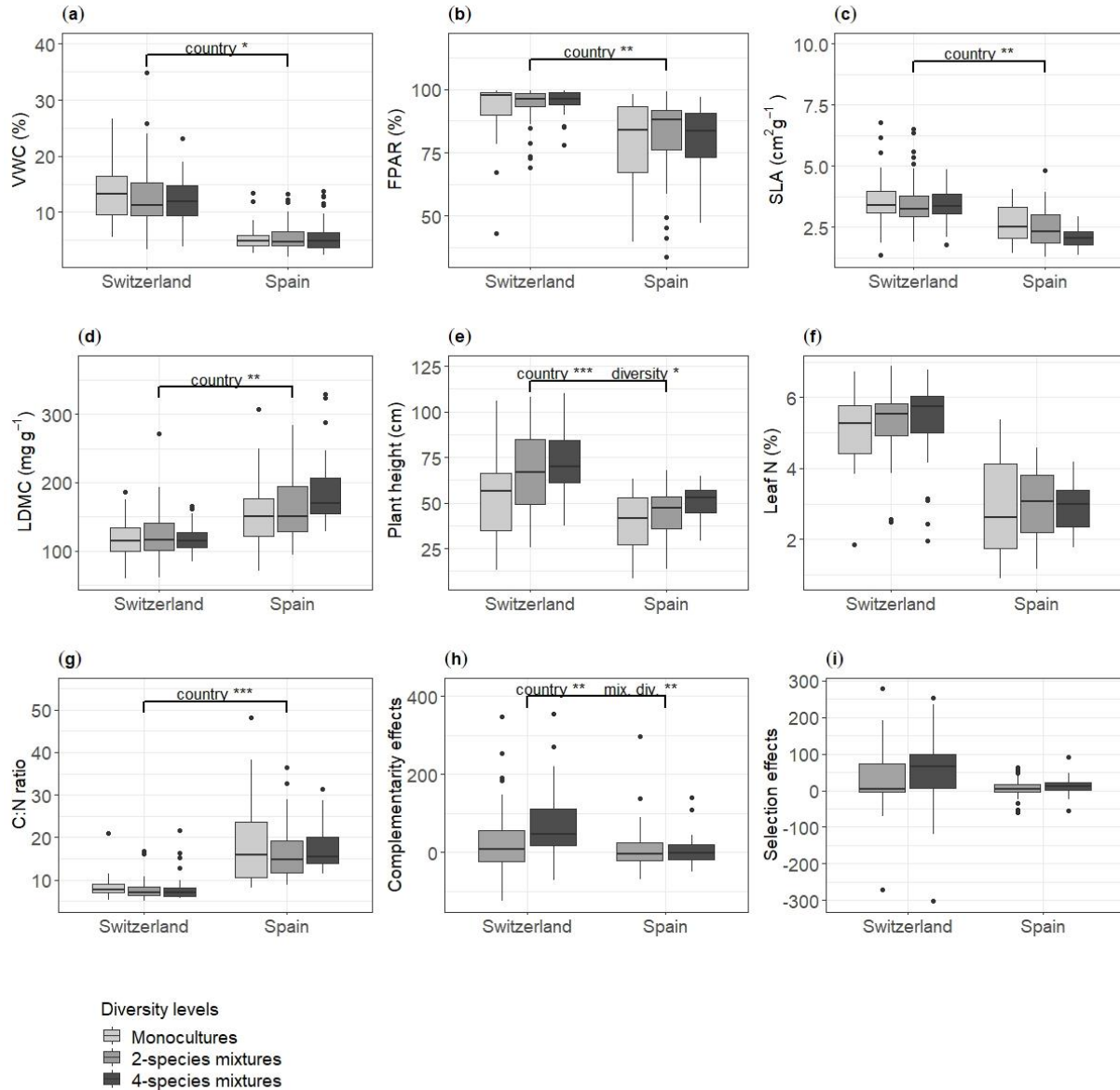
*Figure 1.2: Community-level means for the environmental factors volumetric soil water content (VWC) (**a**) and absorption of photosynthetically active radiation (FPAR) (**b**) and community-weighted means of the plant traits specific leaf area (SLA) (**c**), leaf dry matter content (LDMC) (**d**), plant height (**e**), leaf N (**f**) and C:N ratio (**g**) and the biodiversity effect components, divided into complementarity (**h**) and selection effects (**i**). Data are shown for both countries and separated by levels of diversity. Complementarity and selection effects are only available for mixtures. Brackets indicate significant differences between treatments and labels above brackets indicate which treatment (mix. div. = mixture diversification) was significant at α = 0.05 (* P < 0.05, ** P < 0.01, *** P < 0.001). n = 314.*

1.3.3. Structural equation model to explain community-level yields

The piecewise SEM showed a good fit to the data ($\chi^2 = 38.49$, p-value = 0.357) and explored the links between experimental treatment factors (country, fertilizer, mixture diversification), environmental factors (ΔFPAR, ΔVWC) and the effect of these on the plant traits (ΔSLA; ΔC:N ratio, Δplant height) and finally linked these to the biodiversity effect components, CE and SE (Fig. 1.3).

Biodiversity effect components were negatively correlated to one another. The SE was only related to Δheight, indicating that increasing plant height in mixtures compared to monocultures increased the selection effect (Fig. 1.3). The CE was positively related only to ΔSLA. Increases in ΔSLA indicate larger leaf area per unit leaf dry weight in mixtures compared with monocultures, which increased the complementarity effect.

The negative correlation between ΔC:N ratio and ΔFPAR implies that leaf N content in mixtures increased with increasing light interception. ΔSLA was positively related to both environmental factors, suggesting that increasing light interception and soil water content increased SLA, thus promoting larger leaf area per leaf mass. Based on standardized effect sizes, the effect of ΔFPAR on SLA was stronger than the effect of ΔVWC; specifically, the effect of ΔFPAR on ΔSLA was 1.7 fold stronger than the effect of ΔVWC on ΔSLA. ΔVWC was positively correlated to SE, with the effect of Δheight being 1.2 fold stronger than the effect of ΔVWC on SE. Thus, higher soil water contents in mixtures compared with monocultures increased the SE in mixtures compared with monocultures (Fig. 1.3).

ΔFPAR varied in response to fertilizer treatment, with 10% higher values in unfertilized treatments, indicating that crop mixtures intercepted more light than crop monocultures and that this effect was stronger in unfertilized treatments (Appendix 1: Table A7). ΔVWC varied significantly among countries, with more negative values in Switzerland compared with Spain. This indicates that soils in crop monocultures had a higher water content than in crop mixtures and that this effect was more pronounced in Switzerland than in Spain. ΔC:N significantly varied among countries and was 104% higher in Switzerland than in Spain (Fig. 1.3, Appendix 1: Table A7). Both SE and CE responded significantly to country. CE was 540% higher in Switzerland than in Spain and SE was 350% higher in Switzerland than in Spain. CE was the only variable responding to mixture diversification and CE was 110% higher in 4- than in 2-species mixtures.

The effect of mixture diversification on CE was as strong as the effect from ΔSLA (Fig. 1.3, Appendix 1: Table A7). CE and SE contributed to almost equally to Δyield (Appendix 1: Fig. A5).
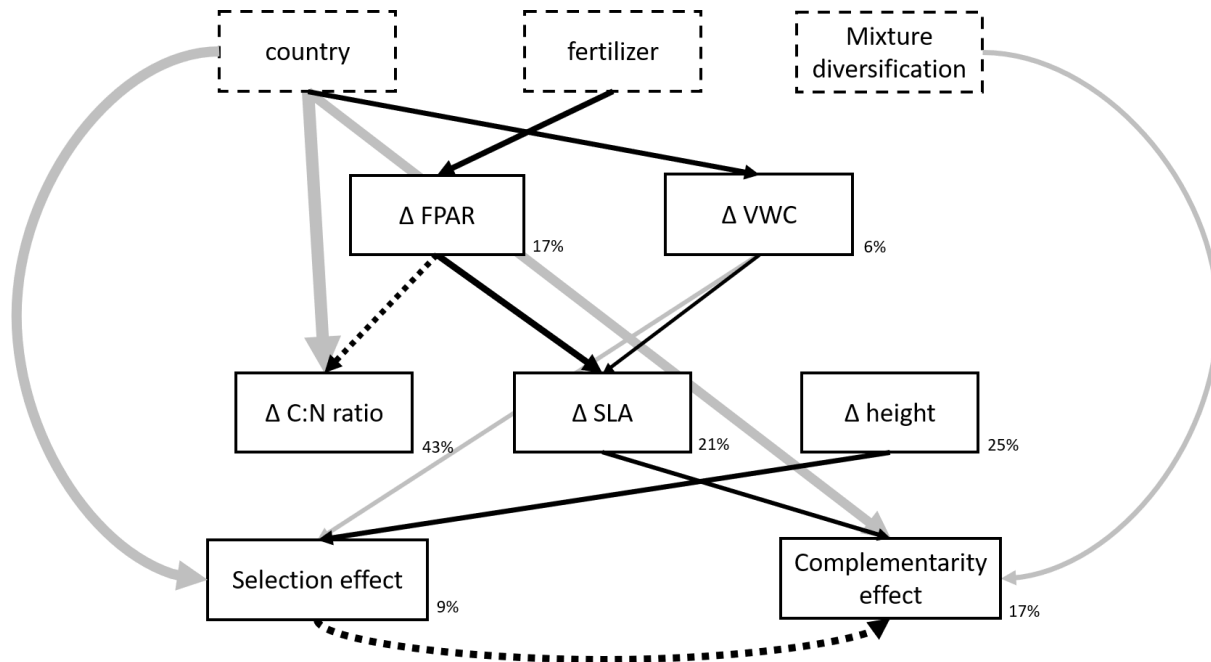
Figure 1.3: Structural equation model showing the effects of experimental treatments on environmental factors on plant traits and on biodiversity effect components. Δ indicates differences between the respective measurements in mixtures compared with monocultures, thus positive Δ values indicate higher values in mixtures compared with monocultures and vice versa. Mean values for Δ values per country, fertilizer treatment and species number are given in appendix 1: table A7. Displayed black arrows show significant positive (solid) or negative (dashed) relationships (α = 0.05), grey arrows indicate direct effects of treatment factors on traits and yield. Arrow thickness indicates effect size based on standardized path coefficients. Numbers next to the variables indicate their explained variance (R^2). Double-headed grey dashed arrows indicate significant correlations. Non-significant tested relationships are not shown. n = 251.

1.4. Discussion

Our study found increasing yields from crop monocultures to 2- to 4-species mixtures at the temperate site in Switzerland but not at the dry site in Spain. Community-level yield did not

respond to fertilizer treatments but varied strongly between the two countries. SE and CE were linked to differences in plant height and SLA, respectively, between monocultures and mixtures. While SLA was linked to light use and soil moisture, plant height showed no link to environmental factors. The effect of mixture diversification on CE was not mediated through any of the abiotic factors or plant traits measured in this study but acted directly upon CE.

Positive biodiversity–productivity relationships are context–dependent

In Switzerland community-level yield increased from monoculture to mixture and from 2- to 4-species mixtures, while diversity showed no effect on yield in Spain. The differences between the two countries were diverse and included differences in precipitation and irrigation, hours of sunshine, soil nutrients, soil carbon and soil texture. Light availability and dry conditions in Spain could have been an inhibiting factor. Lower SLA values in Spain indicate that the plants had less leaf area per dry leaf mass, which could be the plants' effort to reduce leaf area exposed to high irradiance or dry conditions.

From the three growth-limiting resources, soil water and N availability were the most promising to explain the missing positive diversity-productivity relationship in Spain. Soil water content in Spain was kept low by restricting irrigation to the amount needed for plant survival. Combined with a generally drier climate in Spain, the crops were more prone to water stress. Crop yields in intercropping under drought conditions are expected to decrease (Coll *et al.* 2012). Also, positive diversity effects on crop water availability in intercropping remain contested and Brooker *et al.* (2015) suggest that these effects are limited to intercropping systems where at least one species has a low water demand. The crop species planted in this experiment were not adapted to the dry conditions in Spain, since they were Swiss cultivars bred for use under temperate climatic conditions. A further explanation for the absence of a positive diversity-productivity relationship in Spain can be the increased allocation of C to belowground productivity in response to dry conditions. In our study, we were interested in positive productivity effects on crop yield, hence the focus on above-ground biomass. However, increased belowground investment can lead to a decrease in aboveground productivity while maintaining overall community productivity (Kahmen, Perner & Buchmann 2005). Also, available soil water content is an important parameter controlling N_2-fixation of legumes, either directly by influencing nodulation or indirectly by

reducing plant growth and thus N_2-fixation (Sprent & Minchin 1983). However, rather than restricting N_2-fixation in Spain, the higher yield in plots with legumes compared to plots without legumes suggests the presence of a facilitative N sparing effect (i.e. an increased availability of soil N since legumes derived more N from atmospheric N_2 than soil N), which was not visible in Switzerland (Fig. 1.1**b**). However, we propose that this facilitative process was not strong enough to compensate for the difficult growing conditions in Spain. Research has shown that facilitative interactions among plants do not always increase with increasing environmental stress, particularly in arid environments (Maestre, Valladares & Reynolds 2005). These observations can aid the implementation of successful management strategies for mixed cropping systems under stressful environmental conditions, by suggesting that sufficient irrigation and the inclusion of a legume can improve crop growth.

Complementarity effects increased yields in mixtures compared with monocultures

In our study, CE was shown to contribute to positive biodiversity–productivity relationships in both countries (Appendix 1: Fig. A5). The CE was mainly linked to changes in SLA between mixtures and monocultures, implying that crops grown in mixtures were producing a larger leaf area per unit leaf dry mass than crops in monoculture. The increase of SLA in more diverse communities has been observed before and is achieved by an increase in leaf area through the formation of thinner leaves, thus enabling increased light capture, which results in the often observed more complete canopy cover in diverse communities (Williams *et al.* 2020). SLA was dependent on the fraction of intercepted light and on soil water contents. The positive link between SLA and FPAR indicated that plants produced larger leaf areas per unit dry mass when less light was available. In combination with the negative link between C:N ratio and FPAR, this indicates that crops responded to a more complete canopy cover and thus lower light access by increasing their SLA (Fig. 1.3) and leaf N content (lower C:N ratio) to have a larger photosynthetically active leaf area. High leaf N and high SLA are a common plant response to lower light conditions (Reich, Ellsworth & Walters 1998; Evans & Poorter 2001; Funk *et al.* 2017). However, in Switzerland, at higher mixture diversification (i.e. in 4-species mixtures) the relation between FPAR and C:N ratio became positive (Appendix 1: Table A7), indicating that with increasing diversification a shift in N use efficiency occurred. Thus, 4-species mixtures in Switzerland produced more yield per unit N than 2-species mixtures or monocultures. Increasing N efficiency in more diverse communities

has been observed before in semi-natural (van Ruijven & Berendse 2005; Fargione *et al.* 2007) but, to the best of our knowledge, not in intercropping systems.

CE was the only variable responding to mixture diversification, with an increase of CE in 4- over 2-species mixtures. Strikingly, mixture diversification did not affect any of the environmental factors or plant traits measured in this study. This suggests that the plant traits measured in this study were not able to describe how mixture diversification affects complementarity. We suspect belowground processes driving yield increases from 2- to 4-species mixtures or that dynamics of nutrients other than N, or reduced impacts of pests, were possibly playing a role. Concerning possible belowground processes, other studies observed that the presence of a legume can increase N or P availability in the soil surrounding its roots (Temperton *et al.* 2007; Zhang *et al.* 2019). In our study, this observation could potentially be important, as all 4-species mixtures contained one leguminous crop, while not all 2-species mixtures did so. As an alternative to not measuring the appropriate traits, it could also be that not enough traits were measured to fully capture niche differences. As suggested by earlier studies, when linking plant traits to biodiversity effect components, a large range of traits is required to explain niche differences (Kraft, Godoy & Levine 2015; Cadotte 2017).

Selection effects due to Chenopodium quinoa

In this study, SE had a similarly strong effect on yield differences between mixtures and monocultures as CE. While it is often assumed that positive biodiversity–productivity relationships are driven mainly by niche differentiation (Cardinale 2013), we show here that SE was nearly as important. The SE was linked to differences in plant height between mixtures and monocultures, thus SE increased with increasing plant height in mixtures compared to monocultures. A relationship between plant height and SE has been observed before (Cadotte 2017; Li *et al.* 2020) and is probably due to plant height being related to competition for light, where taller plants outcompete shorter plants (Westoby 1998). The observed strong SE in this study could also be akin to the strong SE observed early during a long-term biodiversity experiment, where the SE decreased with time and eventually became negative (Fargione *et al.* 2007).

As the SEM linked the SE to differences in plant height between mixtures and monocultures, we concluded that one highly productive and tall-growing species, *Chenopodium quinoa*, was causing this effect (Appendix 1: Fig. A3). It has been observed before that *C. quinoa* was highly

competitive (Buckland 2016). The significant treatment effect of mixture diversification on plant height, particularly in Switzerland, could represent the increased probability of 4-species mixtures to include a Quinoa crop compared with 2-species mixtures. While half of all 4-species mixtures included a Quinoa crop, only one fourth of all 2-species mixtures did so.

1.5. Conclusion

Our study showed that crop productivity increased with diversity under temperate conditions but only weakly when crops were grown under semiarid conditions with limited availability of water and strong irradiance. Increases in productivity in mixtures compared to monocultures were caused to almost the same extent by complementarity and selection effects. SE and CE were explained by different plant trait syndromes. The SE was maximized in plots with tall plants and was probably caused by one single species, *Chenopodium quinoa*, which was highly productive and tall-growing. CE was linked to increased leaf area per unit weight, indicating that crops in mixtures increased their leaf area to improve light absorption. However, CE was also stronger in 4- compared with 2-species mixtures and this link was not mediated through any of the measured plant traits, suggesting that other ecological processes must have been responsible for the positive diversity effect on yield beyond two-species mixtures. This finding suggests that the drivers of diversity effects from monocultures to mixtures are not the same as from 2- to 4-species mixtures and should therefore be targeted specifically in future studies.

Chapter 2

Temporal differentiation of resource capture and biomass accumulation as a driver of yield increase in intercropping

Abstract

Intercropping, i.e. the simultaneous cultivation of different crops on the same field, has demonstrated yield advantages compared to monoculture cropping. These yield advantages have often been attributed to complementary resource use, but few studies quantified the temporal complementarity of nutrient acquisition and biomass production. Our understanding of how nutrient uptake rates of nitrogen (N) and phosphorous (P) and biomass accumulation change throughout the growing season and between different neighbors is limited.

We conducted weekly destructive harvests to measure temporal trajectories of N and P uptake and biomass production in three crop species (oat, lupin and camelina) growing either as isolated single plants, in monocultures or as intercrops. Additionally, we quantified organic acid exudation in the rhizosphere and biological N_2-fixation of lupin throughout the growing season. Logistic models were fitted to characterize nutrient acquisition and biomass accumulation trajectories.

Nutrient uptake and biomass accumulation trajectories were curtailed by competitive interactions, resulting in earlier peak rates and lower total accumulated nutrients and biomass compared to cultivation as isolated single plants. Different pathways led to overyielding in the two mixtures. The oat–camelina mixture was characterized by a shift from belowground temporal niche partitioning of resource uptake to aboveground competition for light during the growing season. The oat–lupin mixture showed strong competitive interactions, where lupin eventually overyielded due to reliance on atmospheric N and stronger competitiveness for soil P compared to oat.

Synthesis: This study demonstrates temporal shifts to earlier peak rates of plants growing with neighbors compared to those growing alone, with changes in uptake patterns suggesting observed temporal shifts in our experiment were driven by competitive interactions rather than active plant behavior to reduce competition. The two differing pathways to overyielding in the two mixtures highlight the importance of examining temporal dynamics in intercropping systems to understand the underlying mechanisms of overyielding.

Keywords: cumulative biomass accumulation, instantaneous nutrient uptake rates, intercropping, nitrogen, phosphorous, plant–plant interactions, temporal dynamics, root exudates

2.1. Introduction

Intercropping, i.e. the simultaneous growth of two or more species in the same field for all or part of their growing period, is a promising tool to sustainably maintain or increase yields by increasing diversity, thus maintaining natural ecosystem services and thereby limiting the input of agrochemicals (Lithourgidis *et al.* 2011; Brooker *et al.* 2016). Overyielding, i.e. when the productivity of a mixture exceeds the expected yields of the monocultures, in intercropping can occur due to resource complementarity, where two or more species of an intercrop acquire different resources or acquire the same resources at different places belowground (Hauggaard-Nielsen, Ambus & Jensen 2001; Li *et al.* 2018) or at different times (Yu *et al.* 2015; Zhang *et al.* 2017; Dong *et al.* 2018). This reduces niche overlap and competition between individuals in the intercrop. To optimize intercropping systems, the aim is to select crops that differ in resource acquisition in time, space or form to maximize complementarity and reduce competition (Stomph *et al.* 2020).

Recent work has stressed the importance of including temporal dynamics of plant–plant interactions into competition studies (Trinder *et al.* 2012; Zhang *et al.* 2017). While most studies examining the mechanisms underlying dynamic plant–plant interactions have focused on temporal segregation of biomass accumulation, some also include measurements of essential nutrient uptake rates. For instance, Zhang *et al.* (2017) observed temporal niche differentiation between peak N uptake rates in a wheat/barley–maize relay intercropping system, where wheat/barley were sown ~30 days before maize. They observed that maximum N uptake rates of maize when intercropped with wheat/barley were delayed compared to when sole cropped. However, as maize growth was impaired during the co-growth period with wheat/barley (Zhang *et al.* 2015), the observed temporal shift towards later N uptake rates of intercropped maize is likely to be a passive response to early suppression by wheat/barley rather than maize actively changing its N uptake rate to minimize interspecific competition. In contrast, other studies (e.g. Trinder et al. 2012) have shown shifts in the timings of peak uptake rates which cannot be the result of competitive suppression and appear instead to be active responses of plants to neighbors. If plants are able to actively shift the timing of uptake processes so as to avoid competition with neighbors, such responses could

play a key role in increasing niche complementarity and – for crop species – could represent important targets for breeding programmes. However, studies of temporal dynamism of nutrient uptake and biomass are very rare, both in crop and non-crop systems. Therefore, tracking the temporal dynamism of these processes in intercrop systems is extremely valuable in helping us discover plant behavior for niche complementarity and potential mechanisms for yield benefits of intercropping.

Cereal–legume intercrops are usually very effective combinations in intercropping due to complementary N uptake strategies or facilitation of P uptake. The cereal is usually the stronger competitor for soil N and forces the legume neighbor to rely more heavily on atmospherically fixed N_2 (Hauggaard-Nielsen *et al.* 2009), resulting in complementary N use. Moreover, P facilitation has been observed in several cereal–legume intercropping systems such as wheat–lupin and wheat–chickpea, where lupin and chickpea act as the P-mobilizing species (Li *et al.* 2003). By releasing carboxylates into the rhizosphere, the P-mobilizing species can increase the availability of inorganic P in the soil for itself but also for neighboring plant species (Li *et al.* 2014), thereby facilitating P uptake of the neighbor. Hence, complementary processes of both N and P capture are also involved in increasing productivity of cereal–legume intercrops. However, to which extent temporal dynamics in resource acquisition contribute to the success of cereal–legume mixtures is poorly understood. Furthermore, little is known about species mixtures of cereals with non-legumes, particularly cereals intercropped with crop species of the Brassicaceae family. The advantages of intercropping with Brassicaceae species can be multifaceted as Brassicaceae are known to show allelopathic activity, which can potentially be utilized to limit weed pressure, manage crop pests and diseases or even promote crop growth (Rehman *et al.* 2018).

The objectives of this study were to map trajectories of nutrient uptake and crop growth in two different intercropping systems to assess whether i) temporal differentiation in resource uptake and biomass accumulation can explain yield benefits in intercropping systems, ii) temporal differentiation in resource acquisition was reinforced through adjustments of the uptake pattern of species depending on neighbor identity, and iii) intra-specific adjustments in temporal nutrient uptake patterns contribute to yield benefits. Understanding these dynamics will help to improve our ability to predict successful species combinations for intercropping systems and to maximize the advantages of intercropping as a realistic alternative to current monoculture-based agricultural

systems. To achieve this, we intercropped a cereal (oat (*Avena sativa*)) with either a legume (lupin (*Lupinus angustifolius*)) or a Brassicaceae (camelina (*Camelina sativa*)) and also – for comparison of temporal dynamics in inter- and monocrops – cultivated each crop species in a monoculture stand and as isolated single plants. For each species and planting pattern we examined intra- and inter-specific temporal differentiation by comparing maxima of nutrient uptake and biomass accumulation rates and assessed whether (a) intra-specific temporal differentiation increased or decreased inter-specific temporal differentiation and (b) yield benefits. Beyond nutrient uptake and biomass accumulation, we examined some particular physiological processes that could influence nutrient uptake patterns of intercropped species. Specifically, we investigated whether (c) organic acids exudation by the legume or Brassicaceae differed temporally or quantitatively between crop species in intercrops, monocultures and isolated singles and whether (d) it could be linked to increased P uptake of the exuding or neighboring crop species. We expected organic acids exudation of the legume or Brassicaceae to increase P uptake of the neighboring oat. Moreover, we traced biological N_2-fixation of the lupin throughout the growing season to assess whether (e) it differed between lupin intercropped with oat, in monoculture or as isolated single plant. Here, we expected biological N_2-fixation to increase when lupin was intercropped with the cereal, due to increased competition for soil N by the cereal.

2.2. Materials and Methods

2.2.1. Site description

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). The regional climate is classified according to Köppen-Geiger (Kottek et al., 2006) as warm temperate, dry with hot summers. 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 m^2, divided into 480 square plots of 0.25 m^2 which were arranged in 12 beds of 10 x 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. The soil surface beneath the

raised beds consisted of local bare soil and was covered by a penetrable fleece, allowing for root growth beyond 40 cm depth. Each bed on top of the fleece was filled by hand with 40 cm homogenized standard, not enriched, local agricultural soil (Excavaciones Justo Duque, Plascencia, Spain). 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. We are aware that the use of 0.25 m^2 does not reflect a realistic agricultural setting and that plants may experience edge effects. However, using small scale experiments is common in these kind of experiments (Jochum et al. 2020). Furthermore, covering only 120 m^2 of experimental field garden and using the same homogenized soil in all plots at each location allowed us to keep environmental conditions and climatic differences constant throughout one location.

The experimental garden was irrigated throughout the growing season when plants required watering for survival. 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., Switzerland) and the average soil moisture of these six plots defined the soil moisture used for irrigation control. All plots of the experiment received the same amount of irrigation water.

2.2.2. Experimental design

We used a complete randomized block design with three different crop species and three different diversity levels. The crop species were oat (*Avena sativa, cv. Canyon*), lupin (*Lupinus angustifolius, cv. Boregine*) and camelina (*Camelina sativa, cv unknown.*) and the three diversity levels were monocultures, 2-species mixtures and isolated single plants. One block consisted of five plots: one plot of monoculture of each species, one plot with an oat–lupin mixture, one plot with an oat–camelina mixture. The isolated single plants of each species were arranged in a separate block and grown in a different bed to minimize neighbor effects with one plant per 0.25m^2 plot. A monoculture plot consisted of four identical rows of the respective crop species. A mixture plot consisted of two alternating rows of each crop species, following a speciesA|speciesB|speciesA|speciesB pattern. The sowing densities were: 400 seeds/m^2 for oat, 160 seeds/m^2 for lupin and 592 seeds/m^2 for camelina and were based on current cultivation

practice (Olsen, Kristensen & Weiner 2006). A monoculture plot consisted of 4 rows à 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 2-3 February 2019 and sowing depths were 2 cm for oat, 5 cm for lupin and 0.5 cm for camelina.

2.2.3. Sample collection

After seedling emergence, a weekly destructive harvest took place, the first one on 21 February 2019 and the last one on 19 June 2019. Three individuals per plot and per species were randomly selected and marked. Roots of these three individuals were dug out carefully and gently shaken to remove soil. Soil adhering to roots (= rhizosphere soil (Veneklaas *et al.* 2003)) was gently brushed off and collected separately in 15 ml Falcon tubes. Afterwards roots were washed and stored in paper bags. Aboveground biomass of the three individuals was collected separately and separated into leaf, stem and – once available – fruits and seeds. All other individuals per species per plot were counted and aboveground biomass was harvested the same way as for the individuals and then pooled into one sample per species per plot. Aboveground biomass and roots were dried at 75 °C for at least 72h and weighed.

2.2.4. Nutrient analysis

Leaf biomass of the three individuals was pooled and ball milled to powder either in 1.2 ml tubes with two stainless steel beads in a bead mill (TissueLyserII, Qiagen) for three times 5 min or with a mixer mill (Mixer Mill MM 200, Retsch) for 30 seconds. Afterwards, either 100 mg (if available) or 4 mg (if the sample was too small) 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., St. Joseph, USA) and the small (4 mg) samples on a PDZ Europa 20-20 isotope ratio mass spectrometer linked to a PDZ Europa ANCA-GSL elemental analyzer (Sercon Ltd., Cheshire, UK), respectively. All samples of lupin were analyzed on the mass spectrometer to obtain 15N data. Eight samples were cross-referenced on both analytical devices during an earlier study and measured values from LECO were corrected to

account for the differences between the devices (correction factors were 1.0957 for N) (Engbersen *et al.* unpublished).

For P analysis, 100 mg of ground leaf material was weighed into microwave Teflon tubes and 2 ml H_2O_2 (30%) and 1 ml HNO_3 (65%) were added. The samples were digested in a microwave (MLS-1200MEGA ETHOS) for ~25 min at a maximum temperature of 220 °C. The digests were diluted to a sample volume of 10 ml with Nanopure™ water and analyzed for P contents on an ICP-MS (Agilent 7900, Agilent Technologies, USA). For quality control we used the certified WEPAL (Wageningen Evaluating Programmes for Analytical Laboratories) reference materials IPE-100. Nutrient uptake was calculated as the product of nutrient concentration and aboveground biomass.

2.2.5. Organic acids analysis

The rhizosphere soil in the 15 ml Falcon tubes was immersed in 20 ml of a 0.2 mM $CaCl_2$ solution and gently shaken for 30 min. The pH was measured in solution with a portable pH meter (Eutech pH 150, thermo). A subsample of solution was filtered into a 1.5 ml Eppendorf tube through a 0.2 µm syringe filter and acidified by adding a drop of 0.2 M H_2SO_4. All samples were kept frozen until analysis in Switzerland. Samples were analyzed on an IC (940 Professional IC Vario, Methrom) equipped with an ion exclusion column (PRP-X300 Ion Exclusion, Hamilton) and linked to an UV-VIS detector (UV-975, Jasco). The mobile phase was 0.5 mmol l^{-1} sulfuric acid with a flow rate of 2 ml min^{-1}. The UV-VIS detector was connected to the IC with a 771 IC Compact Interface (Methrom) and the wavelength was set to 210 nm. Data processing was performed via MagIC Net Software (Methrom). Identification of organic acids was carried out by comparing retention time and absorption spectra with those of known standards.

2.2.6. Data analysis

All statistical analyses were performed in R version 3.6.0. (R Core Team 2019). To assess crop performance, we calculated the Land Equivalent Ratio (LER), defined as the sum of partial relative yields per species: $LER = \frac{Y_1}{M_1} + \frac{Y_2}{M_2}$, where Y_i is the yield of species i in the mixture, and M_i is the yield of species i in monoculture. Final total plot-level seed yields from the final two harvest weeks

(i.e. weeks 17-18) were used. LER values above 1 indicate a yield advantage of the mixture over the corresponding monocultures (Vandermeer 1989).

Temporal changes in biomass, and N and P accumulation were analyzed by fitting a logistic growth curve using non-linear least squares (nls) models (Trinder *et al.* 2012). Values of biomass, N and P accumulation from crop species in monoculture and mixture from weekly harvests were fitted to the following logistic model:

Eqn. 2.1

$$NU_t = \frac{NU_{max}}{1 + \exp\left(r \times (t_{max} - t)\right)} NU_t$$

NU_t (biomass in g; P, N in mg) is the biomass accumulation or nutrient uptake of a crop species at the number of weeks after seedling emergence (t). NU_{max} (B_{max} for biomass) determines maximum cumulative nutrient uptake (biomass accumulation) of a crop species. r (day^{-1}) is the relative nutrient uptake (biomass accumulation) rate. t_{max} is the time in weeks of reaching maximum nutrient (biomass) uptake rate. Starting values for the nls models were defined by first fitting a nls Levenberg-Marquardt model (nlsLM) and reusing the model parameters as starting values for the nls model. Eqn 2.1 was fitted separately to data for shoot biomass, N and P content across three replicates. Parameters indicating the fit of the models are reported in table A1 in appendix 2.

The instantaneous biomass accumulation (g individual^{-1} week^{-1}) or nutrient uptake (mg individual^{-1} week^{-1}) can be derived as follows:

Eqn. 2.2

$$\frac{dNU_t}{dt} = rNU_t\left(1 - \frac{NU_t}{NU_{max}}\right)$$

The maximum daily biomass accumulation and nutrient uptake rate (g day^{-1}, mg day^{-1}) which emerges at the time t_{max} was calculated as in Zhang *et al.* (2017) and is as follows:

Eqn. 2.3

$$I_{max} = r \times NU_{max}/4$$

Average ^{15}N abundances from the reference plant (oat when available, otherwise camelina) at the same diversity level – i.e. oat from oat–camelina mixture as reference for mixtures, oat from oat monoculture as reference for monocultures and oat single plants as reference for single plants – and same harvest week were used to calculate the proportion of total aboveground lupin N derived from the atmosphere (% Ndfa) on a per plant basis according to equation 2.4:

Eqn. 2.4
$$\%Ndfa = \frac{\delta^{15}N_{ref} - \delta^{15}N_{legume}}{\delta^{15}N_{ref} - \beta} \times 100$$

Where $\delta^{15}N_{ref}$ was the average $\delta^{15}N$ of the reference plant, $\delta^{15}N_{legume}$ the average $\delta^{15}N$ of the lupins and β was obtained in a separate greenhouse experiment. For this, single plants of *L. angustifolius* from the same seed material were grown in 5.5 l pots, filled with 0.7 – 1.2 mm coarse sand and inoculated with 100 ml soil suspension from the field site. Three replicates per harvest week were grown. Pots were watered twice daily with N-free McKnights solution (following the protocol of Unkovich et al. (2008)). Plants were harvested weekly and leaf samples were dried until constant weight, ground and analyzed for ^{15}N as mentioned above. β values for each week are given in table S2.

2.3. Results

2.3.1. Crop performance

Mean LER values (mean ± SE) based on total plot-level grain yield of the final harvest week for the two mixtures were 1.37 ± 0.12 for oat–lupin and 1.14 ± 0.12 for oat–camelina, indicating that both mixtures overyielded. Partial LERs revealed that total plot-level grain yield of oat in both mixtures (oat–camelina: 0.96 ± 0.33, oat–lupin: 0.51 ± 0.0.1) and of lupin in the oat–lupin (0.86 ± 23) mixture exceeded those of oat and lupin in monoculture, respectively. However, camelina in the oat–camelina (0.18 ± 0.06) mixture showed no yield benefits due to intercropping.

2.3.2. Intra-specific variability

No significant differences in biomass accumulation rates or nutrient uptake rates between mixtures, monocultures and isolated singles were observed for any species (Table 2.1), except for biomass accumulation rate of isolated single lupin, which was significantly higher than when the species was grown in a monoculture. However, isolated single plants always showed significantly higher maximum cumulative biomass (B_{max}) and cumulative nutrient uptake (NU_{max}) compared to the respective species grown in a community (i.e. in mixture or monoculture), except for N uptake of camelina (Table 2.1, Appendix 2: Fig. A1).

Table 2.1: Mean values ± SE (n = 3) of model parameters fitted to logistic accumulation curves in Eqn. 2.1 using a nls model for biomass accumulation, N and P uptake. r is the rate constant of biomass production or nutrient capture (d^{-1}), B_{max} is the maximum cumulative biomass (g), NU_{max} is the maximum cumulative nutrient uptake (mg) and t_{max} is the timepoint (number of weeks) at which the maximum biomass accumulation or maximum N or P uptake rate occurred. Parameter estimates with different superscripts within each subsetted column are significantly different between treatments. "Sig." indicates significant differences in t_{max} between the two species intercropped in a mixture and "not sig." indicates no significant differences. Significance is based on non-overlapping SE. Isolated singles of camelina were not fitted as they showed an exponential instead of logistic growth curve.

	Biomass			Phosphorous			Nitrogen		
	B_{max}	r	t_{max}	NU_{max}	r	t_{max}	NU_{max}	r	t_{max}
Oat in oat–lupin	1.62 ± 0.21^a	0.37 ± 0.1	10.48 ± 1.09^a	5.36 ± 0.72^a	1.13 ± 0.82	7.85 ± 0.74	17.45 ± 1.65^a	2.04 ± 1.72	6.72 ± 0.48^a
Oat in oat–cam.	2.45 ± 0.29^b	0.4 ± 0.09	11.59 ± 0.88^{ab}	7.15 ± 0.49^b	1.18 ± 0.43	8.31 ± 0.36	24.9 ± 2.59^b	0.58 ± 0.18	8.95 ± 0.7^b
Oat mono	1.5 ± 0.12^a	0.47 ± 0.11	10.23 ± 0.63^a	7.23 ± 0.92^b	1.3 ± 0.88	8.56 ± 0.61	15.15 ± 1.01^a	0.94 ± 0.33	7.68 ± 0.43^b
Oat single	21.16 ± 4.77^c	0.55 ± 0.25	12.91 ± 1.26^b	38.91 ± 9.89^c	0.68 ± 0.47	9.82 ± 1.34	282 ± 56.41^c	0.61 ± 0.26	11.01 ± 1.05^c
Lupin in oat–lupin	15.26 ± 1.09^a	1.15 ± 0.49^{ab}	9.71 ± 0.43^a	61.26 ± 5.44^a	1.29 ± 0.6	9.61 ± 0.41^a	516.4 ± 46.22^a	1.36 ± 0.73	8.98 ± 0.46^a
Lupin mono	11.35 ± 0.65^b	0.92 ± 0.27^a	9.44 ± 0.38^a	38.7 ± 3.37^b	1.11 ± 0.46	8.95 ± 0.43^a	367.6 ± 26.07^b	1.13 ± 0.45	8.42 ± 0.41^a
Lupin single	126.8 ± 7.37^c	2.18 ± 0.93^b	11.68 ± 0.24^b	33.56 ± 2.49^c	2.49 ± 1.37	11.07 ± 0.2^b	5072 ± 506.8^c	2.12 ± 1.16	11.37 ± 0.32^b
Camelina in oat–cam.	0.52 ± 0.08^a	1.97 ± 2.4	10.6 ± 0.74	2.25 ± 0.55^a	1.37 ± 1.7	9.48 ± 1.04	16.42 ± 4.04	1.38 ± 1.72	10.02 ± 1.06
Camelina mono	0.73 ± 0.11^b	0.68 ± 0.34	11.02 ± 0.91	2.33 ± 0.39^a	1.76 ± 1.83	9.32 ± 0.69	14.61 ± 2	0.91 ± 0.47	9.91 ± 0.7
Camelina single	19.56 ± 3.18^c	0.82 ± 0.55	10.92 ± 0.99	36.02 ± 8.58^b	3.89 ± 18.22	8.76 ± 1.21	NA	NA	16.61 ± 12.12
oat–lupin mixture			not sig.			sig.			sig.
oat–camelina mixture			not sig.			not sig.			not sig.

Oat

Maximum cumulative aboveground biomass of oat intercropped with camelina was 63% and 51% higher compared to oat in monoculture and oat intercropped with lupin, respectively (Fig. 2.1**A**, Table 2.1). Notably, despite biomass gains for oat grown with camelina, maximum cumulative P uptake was not different between oat in monoculture and oat intercropped with camelina but was 33% lower for oat intercropped with lupin (Fig. 2.1**D**). Cumulative P uptake of oat mixed with lupin was similar to that of the other two community combinations until week 8 and then increased more slowly than oat in monoculture and oat mixed with camelina (Fig. 2.1**D**). Cumulative N uptake of oat behaved similarly to biomass accumulation and showed 64% and 43% more accumulated N for oat when intercropped with camelina compared to oat monoculture and oat intercropped with lupin, respectively (Fig. 2.1**G**, Table 2.1). Between weeks 6 and 9, oat intercropped with lupin accumulated N at a faster rate than the other two community combinations, but accumulation came to a halt after week 9 while oat intercropped with camelina continued N uptake. The N uptake rate for oat in monoculture slowed after week 9 and came to a halt after week 11, resulting in the lowest final N accumulation of all combinations (Fig. 2.1**G**, Table 2.1).

Maxima of instantaneous biomass accumulation and nutrient uptake (I_{max}) of isolated single plants were considerably higher than in communities (Appendix 2: Table A3). Maximum instantaneous biomass accumulation of oat grown in mixture with camelina exceeded the maxima of oat grown with lupin by 67% and oat in monoculture by 39% (Fig. 2.2**A**, **B**, Appendix 2: Table A3). The timepoint of maximum instantaneous rates (t_{max}) for biomass accumulation of isolated single oat occurred significantly later than of oat in monoculture and oat mixed with lupin but was not significantly different from oat mixed with camelina (Table 2.1). This indicated that, although not significantly, oat with camelina tended to have a later maximum instantaneous rate of biomass accumulation than oat in the other two community combinations (Fig. 2.2**A**, **B**, Table 2.1). Maximum instantaneous P uptake of oat mixed with camelina and in monoculture was, respectively, 40% and 55% higher than when intercropped with lupin (Appendix 2: Table A3), while no differentiation between the timing of occurrence of maximum rates was observed (Fig. 2.2C, D, Table 2.1). Maximum instantaneous N uptake increased by 146 - 150% when oat was intercropped with lupin compared to when grown in monoculture or mixed with camelina (Fig. 2.2**E**, **F**, Table 2.1, Appendix 2: Table A3). Timepoints of maximum instantaneous uptake rates

of N in oat intercropped with lupin occurred one week earlier than when grown in monoculture and two weeks earlier than when intercropped with camelina. Timepoints of maximum instantaneous N uptake rates of isolated single oat occurred up to four weeks later than when oat was grown in a community (Table 2.1).
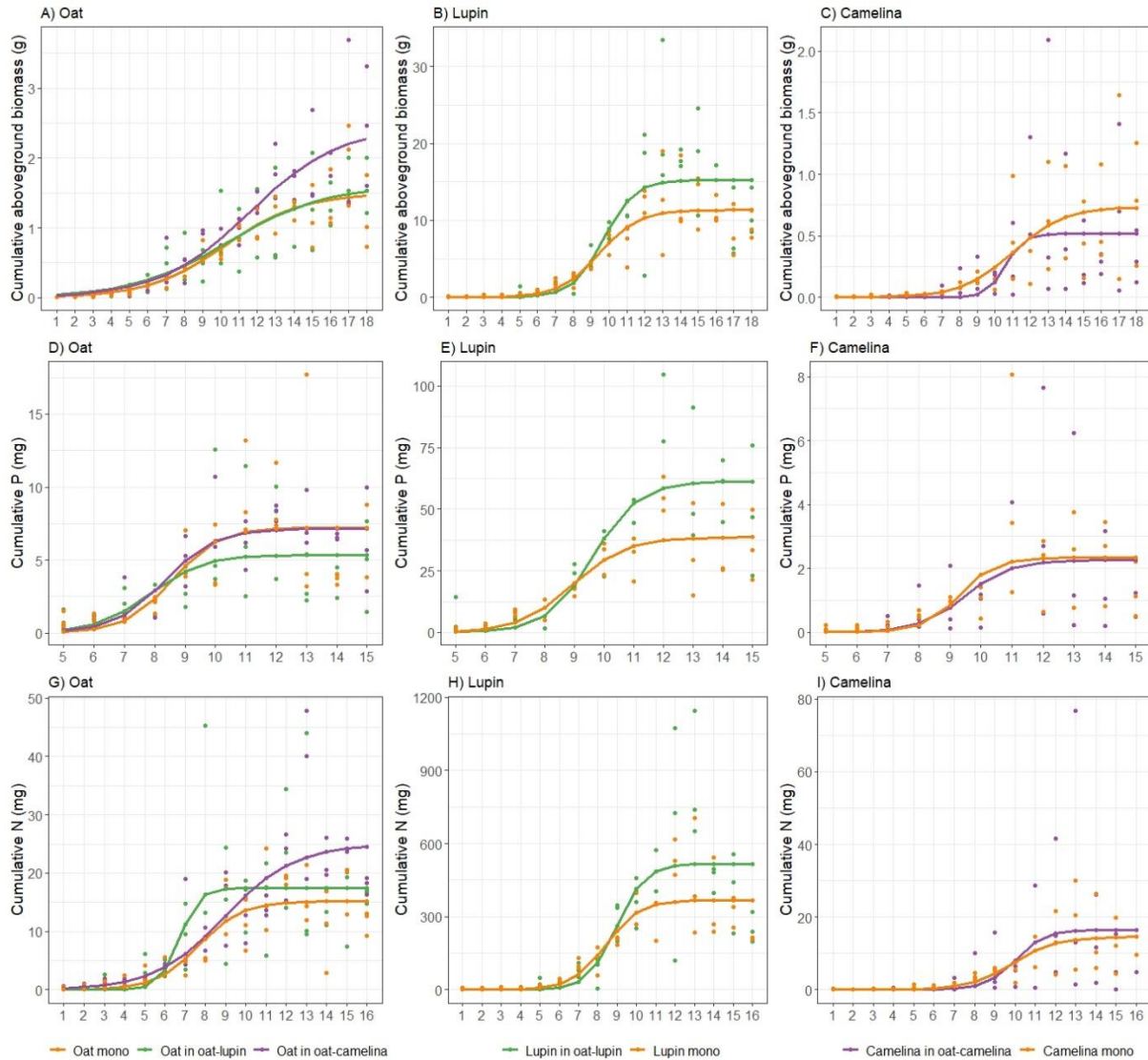
*Fig. 2.1: Trajectories of cumulative aboveground biomass (**A**-**C**), P (**D**-**F**) and N (**G**-**I**) uptake of oat with lupin (**A**, **D**, **G**: green), oat with camelina (**A**, **D**, **G**: purple), lupin (**B**, **E**, **H**) and camelina (**C**, **F**, **I**) when grown in mixture (purple, green) and in monoculture (orange). Curves were derived from Eqn. 2.1. Points show actual data. Note different y-axis scales.*

Lupin

Cumulative nutrient uptake and biomass accumulation of lupin mixed with oat and lupin in monoculture were similar until week 9 and afterwards the mixture outperformed the monoculture. Lupin intercropped with oat accumulated 34% more biomass (Fig. 2.1**B**), 58% more P (Fig. 2.1**E**) and 40% more N (Fig. 2.1**H**) than lupin grown in monoculture (Table 2.1).

Maximum instantaneous biomass accumulation of lupin intercropped with oat was 68% higher than of lupin in monoculture. Similarly, maximum instantaneous uptake of P and N were 84% and 69% higher in intercropped than sole cropped lupin, respectively (Fig. 2.2**A**, **C**, **E**, Table 2.1, Appendix 2: Table A3). No temporal differentiation of maximum instantaneous biomass accumulation or nutrient uptake rates between lupin in monoculture and mixture could be observed. Nevertheless, maximum instantaneous nutrient uptake rates and biomass accumulation of isolated single lupin were always more than 10 times higher and were also 1-2 weeks later than when grown in a community (Table 2.1, Appendix 2: Table A3).

Camelina

Camelina accumulated 40% more biomass in monoculture than intercropped with oat (Fig. 2.1**C**, Table 2.1). While no differences could be observed for cumulative P uptake (Fig. 2.1**F**), camelina tended to accumulate more N when intercropped with oat than in monoculture (Fig. 2.1**I**), although the differences were not statistically significant (Table 2.1).

Maximum instantaneous biomass accumulation and N uptake rates of camelina mixed with oat was 102% and 70% higher than camelina in monoculture, respectively (Fig. 2.2**B**, **F**, Appendix 2: Table A3). Maximum instantaneous P uptake was 33% higher in monoculture camelina than in intercropped camelina (Fig. 2.2**D**, Appendix 2: Table A3). No temporal differentiation of maximum instantaneous biomass accumulation or nutrient uptake rates between camelina in monoculture and mixture could be observed (Table 2.1).
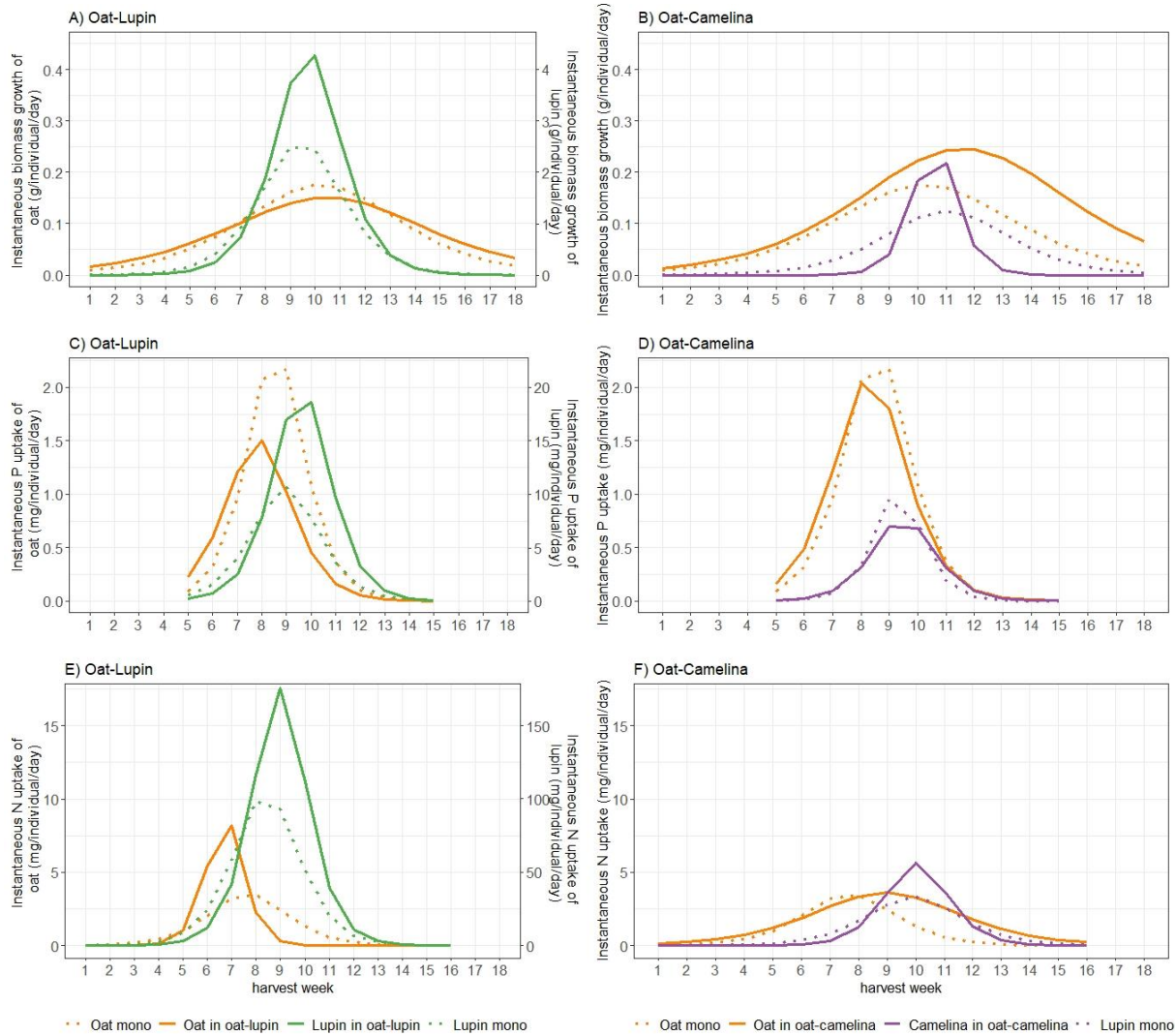
*Fig. 2.2: Instantaneous biomass accumulation (**A**, **B**), P (**C**, **D**) and N (**E**, **F**) uptake of either species in the oat–lupin (**A**, **C**, **E**) and oat–camelina (**B**, **D**, **F**) mixtures (solid lines) and monocultures (dotted lines). Instantaneous biomass growth and N uptake of lupin (**A**, **E**) are given on a second x-axis and are a factor 10 higher compared to values of oat.*

2.3.3. Inter-specific variability

No significant differences in the timepoints of maximum instantaneous nutrient uptake rates in the oat–camelina mixture nor for the timepoints of maximum biomass accumulation rates in both mixtures were observed (Table 2.1). However, maximum instantaneous N and P uptake rates of oat were ~2 weeks earlier than that of lupin in the oat–lupin mixture.

2.3.4. Nitrogen fixation and root exudation

Biological N_2-fixation differed significantly among diversity levels and a post-hoc test revealed that biological N_2-fixation was lower in isolated single lupins compared to lupin grown in mixture or monoculture (Table 2.2, Appendix 2: Table A4). There were no differences in N_2 fixation between harvest weeks for lupin grown in mixture, monoculture or isolated single plants (Appendix 2: Table A4).

Table 2.2: Mean nitrogen derived from the atmosphere (Ndfa)[%] ± SE (n=3) per harvest week for lupin grown in mixture, in monoculture and as isolated single plant.

harvest week	lupin in oat–lupin	lupin in monoculture	lupin as isolated single
	Ndfa (%)		
5	83.06 ± 3.36	77.16 ± 2.33	44.9 ± 3.72
6	76 ± 3	69.33 ± 3.96	43.83 ± 14.43
7	79.41 ± 5.52	70.63 ± 4.26	76.63 ± 13.16
8	78.98 ± 3.46	72.67 ± 6.93	57.69 ± 1.39
9	76.07 ± 1.13	76.91 ± 3.69	57.39 ± 12.23
10	76.51 ± 6.75	74.13 ± 6.39	47.67 ± 12.79
11	72.38 ± 3.56	70.27 ± 8.03	48.6 ± 10.99
12	67.18 ± 10.99	54.03 ± 6.62	38.66 ± 7.99
13	74.1 ± 7.1	66.87 ± 16.63	45.71 ± 7.61
14	76.63 ± 13.16	76.53 ± 6.64	36.91 ± 12.45
15	72.68 ± 6.57	71.21 ± 8.41	37.14 ± 11.14
16	64.92 ± 8.17	70.05 ± 3.66	28.14 ± 5.11

Succinate, malate, acetate, lactate and citrate were detected as organic acids in the crop root exudates. Overall exudation on a per plant basis of organic acids during the entire growing season was highest for camelina followed by oat and lowest for lupin (Fig. 2.3). Although not significant, oat in monoculture tended to exude more organic acids early in the growing season (week 4-7) than when grown in mixture with either lupin or camelina (Fig. 2.3**A**). Organic acid root exudation for lupin was similar whether lupin was grown in monoculture or intercropped with oat and did

not show significant fluctuations throughout the growing season (Fig. 2.3**B**). Camelina intercropped with oat exuded more organic acids and earlier compared to when grown in monoculture (Fig. 2.3**C**). Although camelina exuded larger amounts of organic acids than both, lupin and oat, this higher exudation was not accompanied by a significant drop in rhizosphere pH (Appendix 2: Fig. A2).
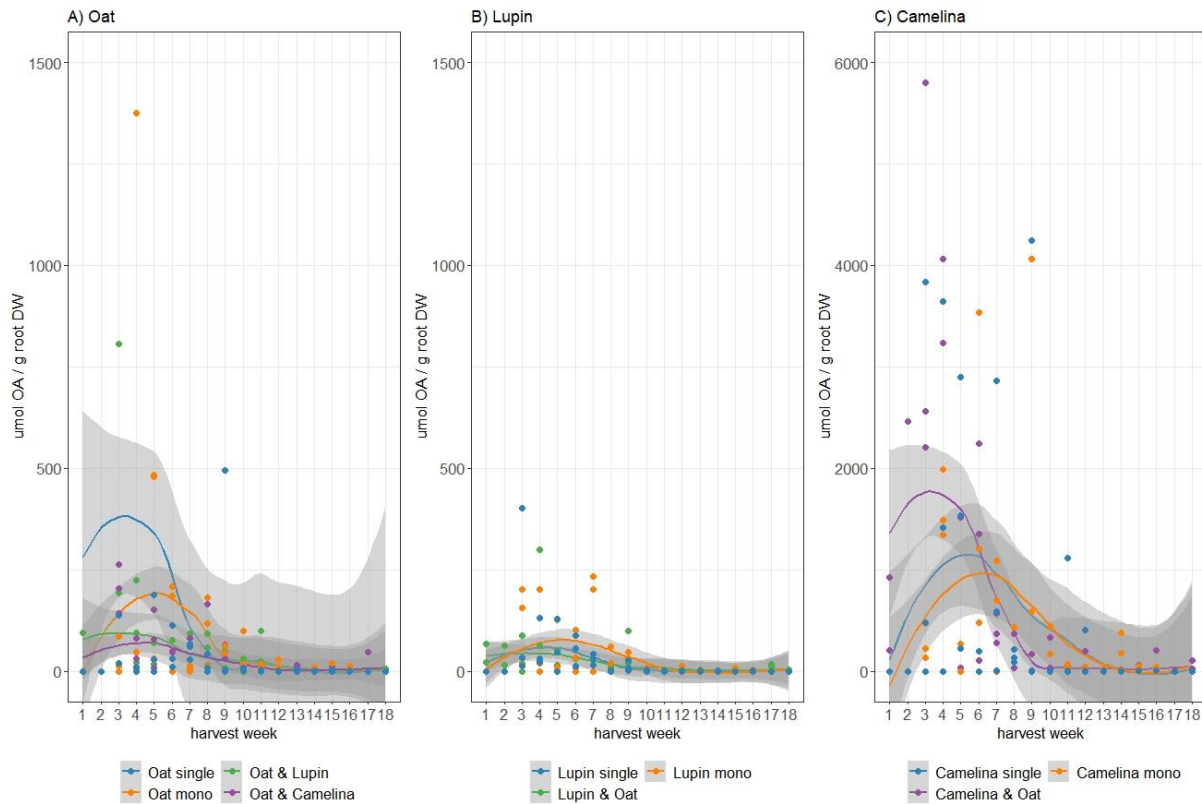
*Fig. 2.3: Total organic acids (OA) found in rhizosphere soil in µmol / g root dry weight of oat (**A**), lupin (**B**) and camelina (**C**) grown as isolated single plants (blue), monocultures (orange) and mixtures (green / purple). Note differing y-axis scales. Shading refers to standard errors computed using a t-based approximation.*

2.4. Discussion

The methodological approach first applied by Trinder *et al.* (2012) allowed us to analyze the dynamics of competitive plant resource capture and biomass accumulation at two different diversity levels and compare them to isolated single plants that did not experience competitive

interactions. We found that competition significantly reduced maximum nutrient and biomass accumulation of plants growing in a community (mixture or monoculture). These plants also showed earlier maximum nutrient uptake and biomass growth, a temporal shift we interpreted as being due to competitive interactions, with uptake and growth trajectories curtailed by competition among and between species.

While both mixtures in this study overyielded, the pathways to overyielding were quite different. Our LER values of 1.37 ± 0.12 for oat–lupin and 1.14 ± 0.12 for oat–camelina were comparable to a LER value of 1.12 ± 0.26 reported in a study using the same sowing densities and experimental setup but a wider range of crop species (Stefan, Engbersen & Schoeb 2021). Based on partial LERs, oat benefited from intercropping and this benefit was stronger when intercropped with camelina than when intercropped with lupin. Lupin benefited from intercropping with oat but camelina did not. In the oat–lupin mixture, oat initially profited from the slower establishment of the lupin and lupin's N coming to 70-80% from biological N_2-fixation. Gradually, however, lupin became a stronger competitor, outcompeting oat for P uptake. Oat N uptake also slowed with time, resulting in a final accumulated oat biomass similar to that in monoculture; while the intercropped lupin strongly overyielded. While the oat–lupin mixture was characterized by strong interactions, the oat–camelina mixture was characterized by temporal partitioning during the early growth stages and aboveground competition in the later growth stages. Here, overyielding of intercropped oat was not due to belowground competitiveness for nutrients, but presumably rather to stronger aboveground competitiveness for light during the later growth stages. Exudation of organic acids did not increase P uptake by oat, although higher exudation by intercropped camelina could have improved N availability via microbial pathways.

Intra-specific variability

The significantly (up to 30 times) higher maximum accumulated biomass of isolated single plants compared to plants in a community, indicated the scale of the effect of intra-specific competition on nutrient uptake and biomass accumulation in crop systems. It is worth acknowledging the increased risk of pest and disease attack in isolated plants (Davis, Radcliffe & Ragsdale 2009). In our case, careful monitoring of isolated plants indicated no signs of enhanced pest or disease attack, and the effect of any such attack would have been to reduce the size of the isolated plants. If this

has happened – and is undetected (which we believe unlikely) – it simply means our estimates of the impacts of competition are relatively conservative.

Isolated single lupins accumulated less N from biological N_2-fixation than lupins in a community. Isolated lupins experienced no competition for soil N, and so might not have invested resources into relatively costly biological N_2-fixation (Vitousek & Field 1999). However, in contrast to other studies (Corre-Hellou, Fustec & Crozat 2006; Hauggaard-Nielsen *et al.* 2008; Hauggaard-Nielsen *et al.* 2009), we observed no difference in N_2-fixation between lupin grown in mixture or monoculture, nor any temporal fluctuations, indicating that lupin in a community relied to a consistent level on biological N_2-fixation.

With respect to temporal shifts, we always found earlier peaks in nutrient uptake and biomass accumulation rates when species were grown in a community. These results contrast with those of Trinder *et al.* (2012) who, in a study using *Dactylis glomerata* and *Plantago lanceolata*, found delay in *D. glomerata* and advancement in *P. lanceolata* of maximum nutrient uptake and biomass accumulation rates when grown in competition. When grown together, the later species, *D. glomerata*, took up more N and suffered less restricted biomass accumulation, indicating it was competitively stronger despite having later peaks in uptake and accumulation rates. In our study, earlier peak rates in a community were always accompanied by significantly lower nutrient and biomass accumulation. Thus, we interpreted the uniform shift towards earlier peak uptake and growth rates for plants in our communities as a passive response to competition, with the onset of competitive interactions between the intercropped species curtailing the trajectories of nutrient uptake and biomass growth, resulting in lower accumulation. This contrasts with the apparently active response found by Trinder *et al.* (2012) where *D. glomerata* shifted uptake and growth to a more favorable timepoint when in competition.

The study of Trinder *et al.* (2012) was conducted with perennial grassland species, and annual crop species might react differently to neighbor presence. For example, a study using two barley cultivars – one early and one late - grown as isolated single plants and in either intra- or inter-specific competition (Schofield *et al.* 2019b) found peak N accumulation was advanced by 0.5 days for the early and delayed by 14.5 days for the late cultivar when in intra-cultivar competition, while no shifts were observed in inter-cultivar competition. This suggests crop species may have enough temporal plasticity to avoid competition with kin but not with other

cultivars. While there have been multiple studies investigating impacts of neighbor density (see e.g. (Rehling, Sandner & Matthies 2021), studies contrasting temporal dynamics between isolated and competing plants are very rare, and so generalities are difficult and the mechanisms behind these processes remain unknown, indicating further research is needed in this area.

Oat–camelina mixture

Oat in monoculture yielded 38% less than in mixture with camelina, despite accumulating similar amounts of P, suggesting P was not a comparatively limiting nutrient in monoculture. However, accumulated N of intercropped oat was significantly higher than monoculture oat. Intercropped camelina accumulated less biomass than camelina in monoculture, but this was not due to nutrient accumulation, which did not differ between camelina in monoculture and mixture (Table 2.1). Thus, we can exclude the idea of oat being a stronger competitor for soil N, which should have resulted in lower N uptake by the intercropped camelina. Instead, the absence of a negative effect of the over-yielding oat on camelina's nutrient uptake could indicate a partitioning of belowground resources during early growth. While this belowground partitioning could have been spatial (i.e. different rooting depths (Kutschera, Lichenegger & Sobotik 2018)), we found some evidence for temporal partitioning, with intercropped oat accumulating N and P earlier than intercropped camelina, translating into an earlier accumulation of biomass by the oat (Fig. 2.2). Earlier accumulation of biomass by the oat could then have resulted in shading of the intercropped camelina, reducing camelina growth later in the growing season. Thus, belowground temporal niche separation could have developed into aboveground competition during later growth stages. Such a shift from a positive, belowground effect early in the season to a negative, late-season aboveground effect has been observed in a study of the interactions between barley and the rare arable weed *Valerianella rimosa* (Brooker *et al.* 2018): early in the season barley had a positive, soil-driven effect on *V. rimosa* abundance but with time this shifted to growth suppression by barley, most likely due to light competition (Brooker *et al.* 2018).

Besides belowground partitioning, exudation of organic acids could have played an additional role in the oat–camelina mixture. Early in the growing season, intercropped camelina exuded almost twice as many organic acids as camelina in monoculture, perhaps improving N availability for both species. In a follow-up study to their (2019b) experiment, Schofield *et al.* (2019a) also

examined temporal dynamics of soil microbial enzyme activity in the same system and found that temporal dynamics of plant resource capture and soil microbial activity were linked. However, while root exudates can influence soil N availability via microbial pathways (Meier, Finzi & Phillips 2017), root-microbe-soil nutrient interactions are complex, and our understanding of the exact mechanisms is limited (Zhang, Vivanco & Shen 2017).

Oat–lupin mixture

The observed yield advantage for oat in the oat–lupin mixture agrees with often-observed complementarity in resource use or facilitation in cereal–legume mixtures (Duchene, Vian & Celette 2017). We observed that oat intercropped with lupin initially accumulated N at a faster rate than oat grown in other combinations, which is explained by the slow establishment of lupin (Fig. 2.1) and the general observation that biological N_2-fixation in lupin only starts four to five weeks after emergence, after which N does not accumulate in shoots for two further weeks (Walker *et al.* 2011). Perhaps because biological N_2-fixation is a P-demanding process (Walker *et al.* 2011), we observed a close link between the instantaneous N and P uptake rates of intercropped lupin (Fig. 2.2) and a strong competitiveness of intercropped lupin compared to oat for soil P, resulting in lower accumulated P in lupin–intercropped oat (Fig. 2.1). However, despite reductions in P uptake, the biomass of lupin–intercropped oat was not negatively affected, and it still accumulated more N than in monoculture. We suggest this latter effect was due to lupin capturing ~70-80% of its N from biological N_2-fixation.

Surprisingly, N accumulation of oat intercropped with lupin stopped after week nine, even though lupin captured N from biological N_2-fixation throughout the experiment. There are two possible explanations. First, intra-specific competition among oats could have limited N accumulation at that point in time. Intra-specific competition for N is also visible in the oat monoculture and, although occurring slightly later than in the lupin-intercropped oats, it results in lower final N accumulation, an effect which may be explained by intercropped oats having the advantage of growing with a slowly-establishing legume. The second explanation is that after slow establishment, lupin rapidly accumulated biomass which shaded the oats and reduced N uptake. As N accumulation is closely related to photosynthetic capacity (Sinclair & Horie 1989), reduced N uptake may reflect limited photosynthetic capacity of oats due to light competition with lupin.

However, from our data we cannot differentiate between the two. Alternatively, the seemingly drop in N accumulation may also be explained by nutrient dilution, where nutrient concentrations tend to decrease with increasing plant biomass due to relocation of the nutrient from the leaf to reproductive tissues (Jarrell & Beverly 1981).

2.5. Conclusion

This study shows how temporal dynamics of resource uptake and biomass accumulation throughout the growing season can shed light on competitive plant–plant interactions and improve our understanding of the underlying processes that drive yield advantages in intercropping systems. We showed that trajectories of nutrient uptake and biomass accumulation were curtailed by competition, leading to earlier peak rates and lower total accumulated nutrients and biomass. Our results also revealed multiple pathways to overyielding. We observed strong competitive interactions in the oat–lupin mixture, but the oat–camelina mixture was characterized by an apparent shift from positive, belowground temporal partitioning early in the growing season to aboveground competitive interactions later in the growing season. While this study focused on the temporal dynamics of nutrient uptake, biomass accumulation, biological N_2-fixation and root exudation, our results suggest that temporal patterns of aboveground competitive interactions for light could be equally important for understanding plant–plant interactions in intercropping systems. Understanding temporal dynamics of below- and aboveground resource uptake and biomass accumulation in intercropped plant communities will enable us to maximize the benefits from intercropping systems, as it will facilitate the optimization of these systems with respect to nutrient inputs, enabling us to better design efficient species combinations where temporal differentiation can reduce competition between species and thus increase yields.

Chapter 3

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

Abstract

Intercropping systems can be more productive than their respective monocultures and this positive net biodiversity effect is caused by complementarity and selection effects. 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.

We conducted weekly destructive harvests to examine temporal dynamics of biodiversity effects in two intercropping systems (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.

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, i.e. increased photosynthetic capacity and increased photosynthetic efficiency contributed to a positive net biodiversity effect in the oat–lupin and oat–camelina mixture, respectively.

By linking the temporal dynamics of the net biodiversity effect and its two additive components to light-use related traits in two different intercropping systems, this study contributes to a better understanding of the underlying mechanisms that contribute to overyielding in intercropping systems.

Key words: intercropping, crop mixtures, light use, plant traits, temporal dynamics, biodiversity effects, complementarity effect, selection effect

3.1. Introduction

Intercropping, where at least two crop species are cultivated on the same field at the same time, aims to sustainably increase yields through improved resource capture and lower artificial inputs (Vandermeer 1989). Complementary resource use is considered a driving force for positive biodiversity effects in diverse plant communities as it decreases the niche overlap between species and thus reduces competition (Brooker *et al.* 2015). This resource partitioning can occur above- and belowground and minimizes the niche overlap between species and thus enables an increased resource capture in the intercrop compared to the monoculture. While many studies have observed

partitioning of belowground resources (Hauggaard-Nielsen, Ambus & Jensen 2001; Bedoussac & Justes 2010; Li *et al.* 2018), evidence that these processes contribute to positive biodiversity effects remains limited (Barry *et al.* 2020). This suggests that complementary use of light might be an important but, to date, overlooked mechanism driving increased productivity in diverse communities (Yang *et al.* 2017; Jesch *et al.* 2018).

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 (Zhang *et al.* 2008). The increased light interception in intercrops comes at the cost of shading, where shorter crops suffer shading from taller crops (Lv *et al.* 2014). 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 adaptions encompass – among others – increased specific leaf area (SLA) (Niinemets 2016), increased photosynthetic capacity (Franco, King & Volder 2018) or photosynthetic efficiency (Gong *et al.* 2019). Photosynthetic capacity describes the maximum rate at which a leaf is able to fix C and has been tightly associated with leaf N content, since the photosynthetic machinery accounts for approx. half of the leaf N content (Evans 1989). For instance, as a response to lower light conditions in the intercrop, leaf N content was observed to increase in watermelon when intercropped compared to when cultivated in monoculture (Franco, King & Volder 2018). Photosynthetic efficiency describes the efficiency by which captured light is converted into biomass (Long et al. 2006). Photosynthetic processes are known to be highly sensitive to shading and plants can adapt their photosynthetic characteristics to various light environments (Huang *et al.* 2011), as shown in a recent study where an increased efficiency of photo system II (PS$_{II}$) in proso millet was observed in response to being intercropped (Gong *et al.* 2019).

Thus, although increased light interception in intercrops comes at the cost of increased shading, plants have evolved to offset the negative effects of increased shading by adapting their photosynthetic processes to the lower light conditions. Thanks to these adaptions, intercropping systems can enable positive light-driven biodiversity effects. However, even though many studies have examined light use in intercropping systems, the relative extent to which complementary use of light contributes to positive biodiversity effects in intercropping is poorly understood (Zhu *et al.* 2015). 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) (Loreau & Hector 2001). Based on work from semi-natural grasslands, it was found that the complementarity effect was the main contributor to overyielding in diverse mixtures and that complementarity effects were largest in mixtures containing species of different functional groups (Cardinale 2007; Huang *et al.* 2020). In line with these results, a recent meta-analysis examining the contribution of CE and SE to overyielding in Chinese relay intercropping systems found that the CE caused 90% of yield gains (Li *et al.* 2020). More generally, species from different functional groups differ in uptake patterns, traits and life history and are therefore more likely to differ in their niches (Marquard 2009). However, increased productivity in diverse communities is not caused by complementarity alone but is also driven by highly competitive species that contribute strongly to higher yields in mixtures. This selection effect was found to be related to plant height, where communities with tall plants and lower overall trait diversity had stronger selection effects (Cadotte 2017; Engbersen *et al.* unpublished). Distinguishing whether positive biodiversity effects are driven by complementarity or selection effects 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 net biodiversity effect can change over time (Fargione *et al.* 2007). Lately, studies examining temporal dynamics of plant interactions have gained popularity, as they have unraveled important processes preceding the harvest that would have gone by unnoticed if not detected through a series of destructive harvests (Trinder *et al.* 2012) and have generally contributed to a better understanding of dynamic processes in diverse plant systems (see e.g. Zhang *et al.* 2017, Dong *et al.* 2018, Engbersen *et al.* 2021a). While earlier studies have shown that the amount of intercepted light increases during the growing season (Kanton & Dennett 2008), to the best of our knowledge, there are no studies that examined temporal changes of light-use associated traits in annual intercropping systems.

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 intercropping 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. We hypothesized that biodiversity effects would increase with time and that complementarity effects contribute more to the net biodiversity effect than selection effects. We further hypothesized that the SE would be mainly correlated with plant height, indicating competitiveness in light acquisition of a highly productive species, while the CE would contribute to increased NE via a range of different traits related to light use. 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 complementarity effects were expected to be particularly strong in mixtures with crops from differing functional groups, we combined oat (*Avena sativa)*, a grass, with either a legume (lupin, *Lupinus angustifolius*) or a Brassicaceae (camelina, *Camelina sativa*).

3.2. Methods

3.2.1. Site description

The site and experimental design are identical to the one used in Engbersen *et al.* (2021a). 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 m^2, divided into 480 square plots of 0.25 m^2 which were arranged in 12 beds of 10 x 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. We are aware that the use of 0.25 m^2 plots does not reflect a realistic agricultural setting and that plants may experience edge effects. However, covering only 120 m^2 of experimental field garden and using the same homogenized soil in all plots at each location allowed us to keep environmental conditions homogeneous across the whole experiment, and using small scale plots is not uncommon in these kind of experiments (Jochum *et al.* 2020).

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., Switzerland) and the average soil moisture of these six plots defined the soil moisture used for irrigation control.

3.2.2. 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. The sowing densities were: 400 seeds/m^2 for oat, 160 seeds/m^2 for lupin and 592 seeds/m^2 for camelina and were based on current cultivation practice (Olsen, Kristensen & Weiner 2006). A monoculture plot consisted of 4 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 2-3 February 2019 and sowing depths were 2 cm for oat, 5 cm for lupin and 0.5 cm for camelina.

3.2.3. 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 being 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 72h 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 three times 5 min or with a mixer mill (Mixer Mill MM 200, Retsch) for 30 seconds. 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., St. Joseph, USA) and the small (4 mg) samples on a PDZ Europa 20-20 isotope ratio mass spectrometer linked to a PDZ Europa ANCA-GSL elemental analyzer (Sercon Ltd., Cheshire, UK).

3.2.4. Light measurements

Photosynthetically active radiation (PAR) was measured with a LI-1500 (LI-COR Biosciences GmbH, Germany) 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 (ΦPS_{II}) was measured with a fluorometer (MINI-PAM, Walz, Germany) equipped with a dark leaf clip (DLC-8). ΦPS_{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 (Genty, Briantais & Baker 1989; Maxwell & Johnson 2000). 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 (12000 µmol photons m^{-2} 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 ΦPS_{II} indicate a reduced quantum efficiency of photosynthesis, indicating that plants are increasingly stressed. Values above c. 0.7 are considered normal for healthy plants (0.83 corresponds to maximal efficiency), whereas values below c. 0.7 indicate stress.

3.2.5. Data analyses

To explain differences in community-level yield between mixtures and monocultures, we quantified the net biodiversity effect (NE) and its two additive components, the complementarity effect (CE) and selection effect (SE) according to Loreau and Hector (Loreau & Hector 2001):

$$\Delta yield = N \cdot \overline{\Delta RY} \cdot \overline{M} + N \cdot cov(\Delta RY, M) \qquad (3.1)$$

Where N is the number of species in the plot. ΔRY is the deviation from expected relative yield of the species in mixture in the respective plot, which is calculated as the ratio of observed relative yield of the species in mixture to the yield of the species in monoculture. M is the yield of the species in monoculture. The first component of the net biodiversity effect equation ($N \cdot \overline{\Delta RY} \cdot \overline{M}$) is the CE, while the second component ($N \cdot cov(\Delta RY, M)$) is the SE. Yield refers to total aboveground biomass for the harvest weeks 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). Harvest weeks 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.

Since the net biodiversity effect 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, Δ height was calculated as:

$$\Delta height = \overline{height}_{mix} - \overline{height}_{mono} \qquad (3.2)$$

Where $\overline{height}_{mix}$ is the average of all three measurements of height per mixture plot and $\overline{height}_{mono}$ the average of all three measurements of height of the respective monoculture plot. Weights for community-weighted means were 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. (R Core Team 2019). 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) ANOVA . The fixed effects of the model were the differences between mixtures and monocultures of each light-use associated trait (FPAR, plant height, ΦPS_{II}, leaf N) and the interactions between each of these with harvest week (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 put back on the transformed values for analysis (Loreau & Hector 2001). We tested for correlation among the light-use associated traits using Pearson's correlation coefficient. If traits strongly 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).

3.3. Results

3.3.1. Biodiversity effects

Biodiversity effects were based on total aboveground biomass during the vegetative period (i.e. harvest weeks 1-14) and on total grain yields during the reproductive period (i.e. harvest weeks 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 3.1,

Fig. 3.1 **A-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 mix. × HW in Table 3.1, Fig. 3.1**B**). During the reproductive phase, the NE (Fig. 3.1**D**) and CE (Fig. 3.1**E**) were significantly higher in the last compared to the second last harvest week, continuing the same trend of an increase of NE and CE with time. The selection effect did not increase with time but was significantly higher in the oat–lupin compared to the oat–camelina mixture (Fig. 3.1**F**).
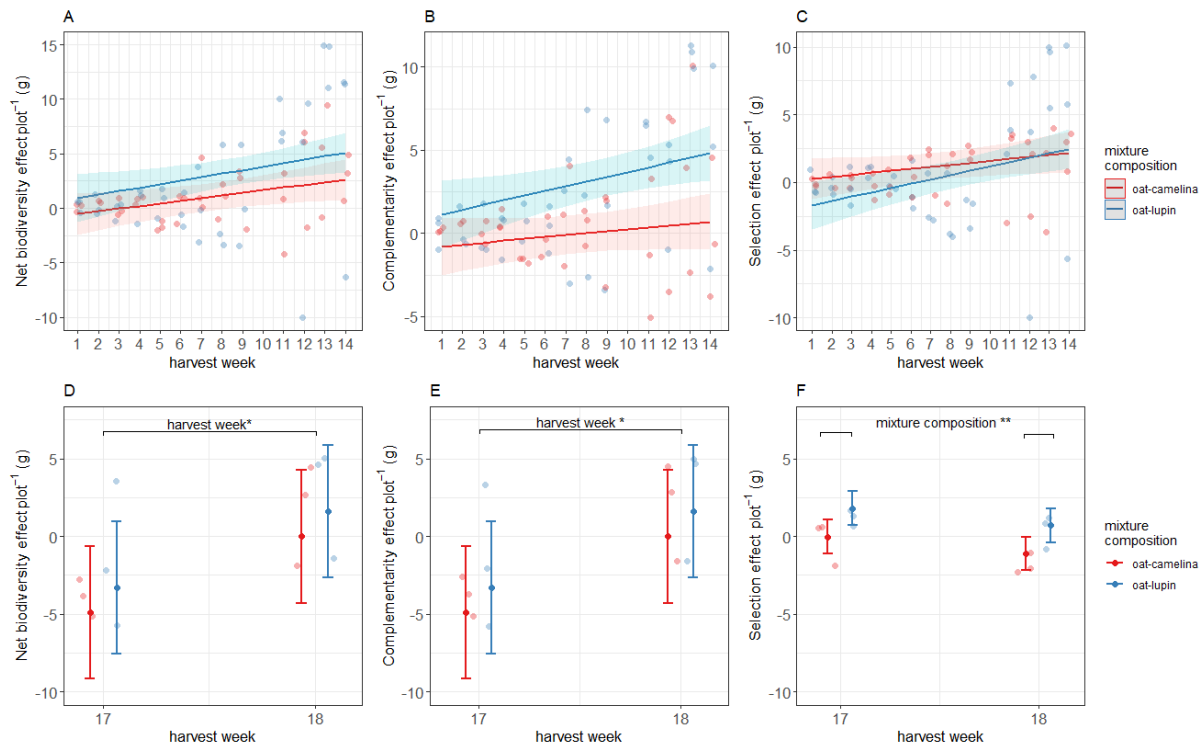
Fig. 3.1: The net biodiversity effect (A, D), complementarity effect (B, E) and selection effect (C, F) based on total biomass for the vegetative period (A-C) (i.e. harvest weeks 1-14) and based on total grain yields for the reproductive period (D-F) (i.e. harvest weeks 17-18) shown for oat–camelina (red) and oat–lupin (blue) mixtures. Lines in A-C show the marginal effect associated with the linear model presented in Table 1. Data in D-F are mean and 95% CI and significance analyses are based on linear models presented in Appendix 3: Table A2.

3.3.2. Light-use associated traits and biodiversity effects

Collinearity among the light-use associated plant traits occurred between Δ diameter and Δ height (Appendix 3: Table A1). Model comparison based on Akaike information criterion (AIC) indicated that the model fit improved after removing Δ diameter as explanatory variable from the model.

Δ FPAR

Increases in Δ FPAR significantly increased with NE, CE and SE (Table 3.1, Fig. 3.2**A**, 3.3**A**, 3.4**A**) 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 3.1). 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.

Δ Height

Overall, all three biodiversity effects increased with Δ height (Table 3.1, Fig. 3.2**B**, 3.3**B**, 3.4**B**). In the oat–lupin mixture, the SE decreased with increasing Δ height during the earlier harvest weeks but the relationship became positive during the later growing season (Fig. 3.4**B**). In the oat–camelina mixture, the relationship between SE and Δ height changed from positive in the early growing season to negative in the later growing season (Fig. 3.4**B**). 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 (Fig. 3.3**B**).

Δ Efficiency of PS_{II}

The interaction Δ ΦPS_{II} × mix. × HW (Table 3.1) was significant only for the CE, indicating that in the oat–camelina mixture, the relationship between CE and Δ ΦPS_{II} became positive and stronger over time, while in the oat–lupin mixture the relationship remained largely neutral during the entire season (Fig. 3.3**C**). These results indicated that efficiencies of PS_{II} were comparable between crops in mixture and monoculture in the latter system.

Δ Leaf N

Δ leaf N showed an overall positive relationship with NE in the oat–lupin mixture and an overall negative effect with NE in the oat–camelina mixture (Fig. 3.2**D**). Over time, the effect became stronger, i.e. more positive in the oat–lupin mixture (interaction Δ leaf N × mix. × HW in Table

3.1, Fig. 3.2**D**). Δ leaf N was negatively correlated to SE in the oat–camelina mixture and positively in the oat–lupin mixture (interaction Δ leaf N × mix. in Table 3.1, Fig. 3.4**D**). 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 × mix. × HW in Table 3.1, Fig. 3.4**D**). No effect of Δ leaf N was observed on CE (Table 3.1).

Table 3.1: 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). 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 (harvest weeks 1-14). SS: Sum of squares, F-value: variance ratio, P: error probability. P-values in bold are significant.

		NE			CE			SE		
	Df	SS	F-value	P	SS	F-value	P	SS	F-value	P
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**	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 ΦPS_{II}	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
ΔFPAR × 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 ΦPS_{II} × 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
ΔFPAR × 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 ΦPS_{II} × 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
ΔFPAR × 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 ΦPS_{II} × 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									
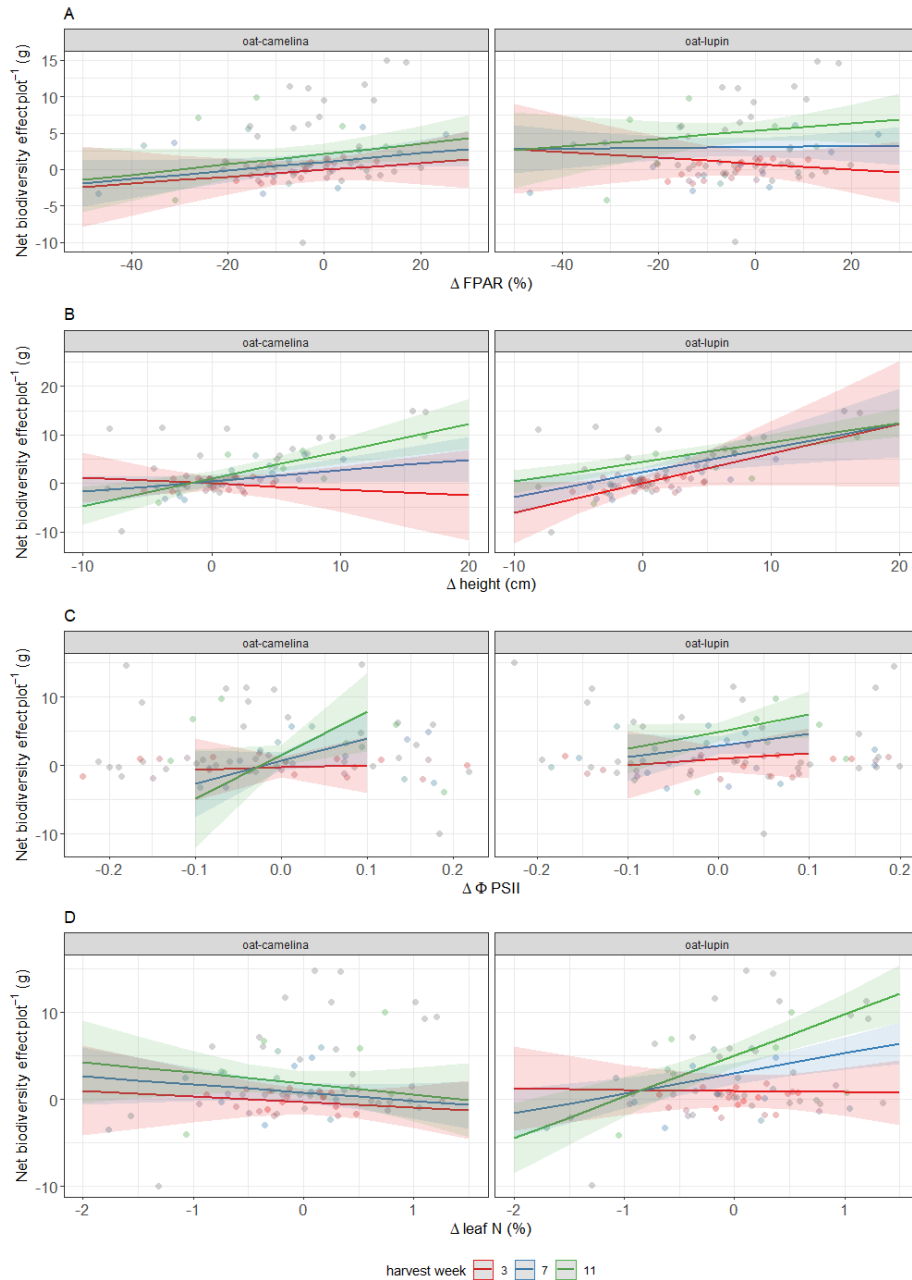
Fig.3. 2: Relationships between net biodiversity effect (NE) and A) differences in FPAR between mixtures and monocultures (Δ FPAR), B) differences in height between mixtures and monocultures (Δ height), C) differences in the efficiency of PSII between mixtures and monocultures (Δ ΦPSII) and D) differences 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% CI associated with the linear model (Table 3.1).
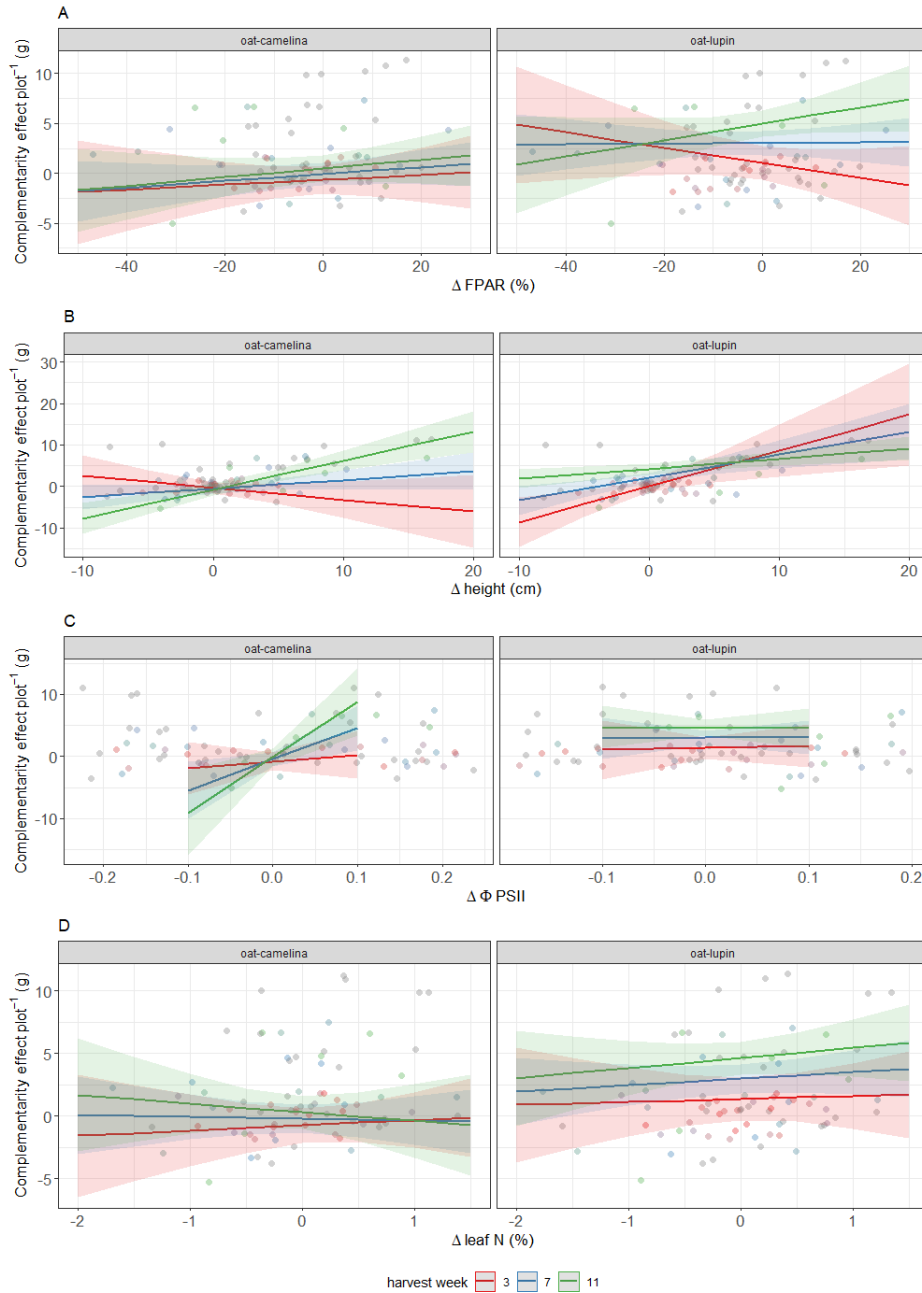
Fig. 3.3: Relationships between complementarity effect (CE) and A) differences in FPAR between mixtures and monocultures (Δ FPAR), B) differences in height between mixtures and monocultures (Δ height), C) differences in the efficiency of PSII between mixtures and monocultures (Δ ΦPSII) and D) differences 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% CI associated with the linear model (Table 3.1).
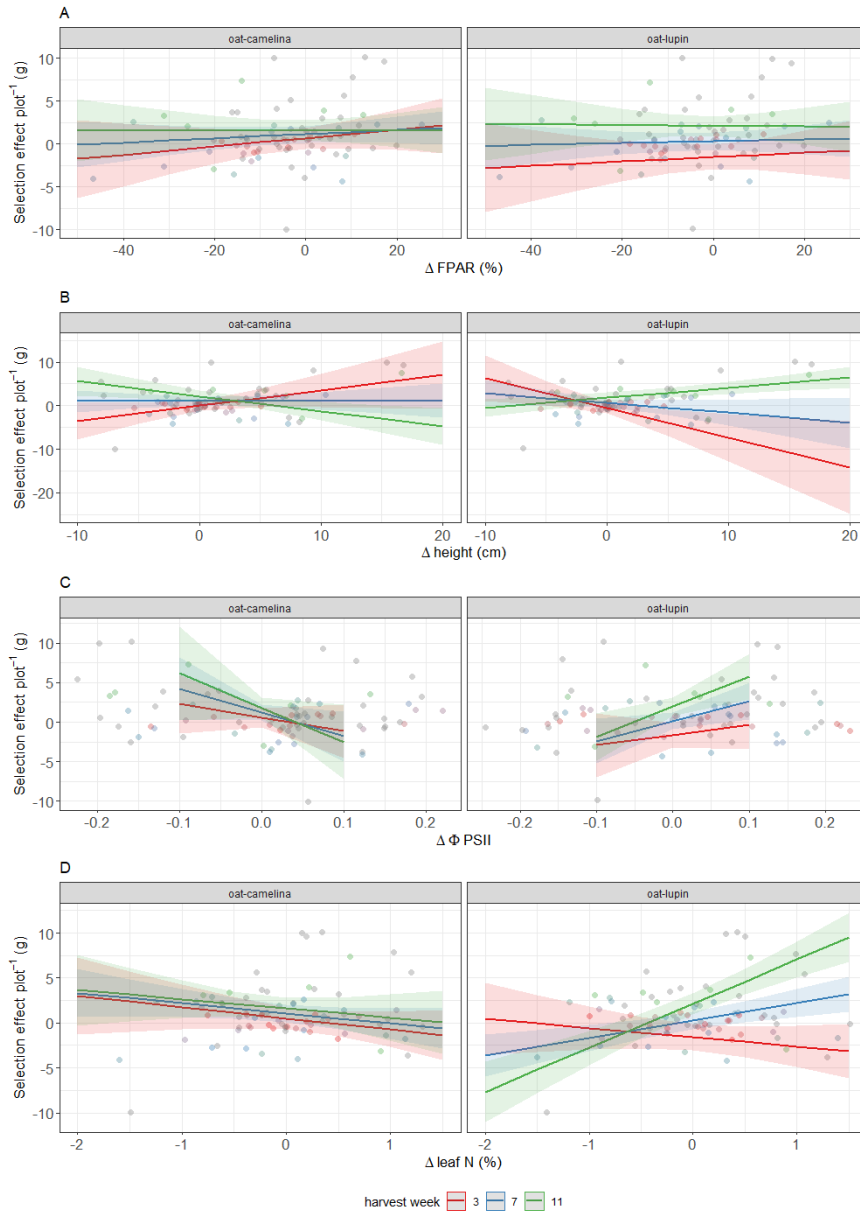
Fig. 3.4: Relationships between selection effect (SE) and A) differences in FPAR between mixtures and monocultures (Δ FPAR), B) differences in height between mixtures and monocultures (Δ height), C) differences in the efficiency of PSII between mixtures and monocultures (Δ ΦPSII) and D) differences in 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% CI associated with the linear model (Table 3.1).

3.4. Discussion

This study found increasing net biodiversity and selection effects in two different crop mixtures over time during the vegetative period. Complementarity effects were found to increase only in the mixture containing a legume. While the NE and CE also increased during the reproductive period, the SE did not. This could suggest a discrepancy between the effects of biodiversity on biomass and seed yield.

We found that higher light interception in mixtures compared to monocultures contributed positively to the net biodiversity effect 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 PS_{II} contributed to the CE in the oat–camelina mixture, an increased photosynthetic capacity contributed to the SE in the oat-lupin mixture.

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 decades (Isbell *et al.* 2018; Qiu & Cardinale 2020). On a shorter time-scale, a recent study has shown that relative contributions of the selection and complementarity effect to the net biodiversity effect changed over the course of a year in a grassland mixture (Mason *et al.* 2020). 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 net biodiversity effect 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 (Fargione *et al.* 2007). 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 (Chen *et al.* 2021). Increasing complementarity effects 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 (Duchene, Vian & Celette 2017). Most complementarity effects in cereal-legume mixtures are soil-driven, and are attributed to the legume meeting most of its N demand by fixing atmospheric N_2 and thus leaving most soil N for the neighboring cereal, which has been observed before for oat-lupin mixtures (Engbersen *et al.* 2021a). However, the present study could also show that specifically for the oat-lupin mixture, complementarity in light-use thanks to the differences in canopy architecture between the intercropped species could further contribute to complementarity in this mixture.

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 in 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 thanks to complementarity in plant architecture between different species (Spehn *et al.* 2000). 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 thanks to species-specific differences in morphology and increase complementary light use in mixtures (Tremmel & Bazzaz 1993; Spehn *et al.* 2000). In our study, both mixtures consisted of crop species from different functional groups with quite different canopy structures. Oat, a grass, was characterized by mainly erect leaf surfaces, permitting a substantial amount of light to reach the ground unused. The other two species, a Brassicaceae and a legume, had more horizontal leaf surfaces and therefore also intercepted more light.

Contributing to more complex canopy structures was also the taller growth of plants when grown in mixture compared to monoculture, which supported the complementarity effect. 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 (Fig. 3B). Increased plant height is an indicator for light competition, as growing tall improves the plants' access to light and expresses competitive ability over neighbors (Falster & Westoby 2003; Gommers *et al.* 2013). Our observations of increasing plant height in mixtures compared to monocultures are in line with other studies, who found that plants grew taller in maize–soybean mixtures compared to the respective monocultures (Liu *et al.* 2017) and this could even be linked to yield increases of the intercrop (Hanming *et al.* 2012). 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 (Engbersen *et al.* 2021a).

In contrast to positive effects of Δ height on CE, differences in height between mixtures and monocultures were negatively related to SE, particularly in the later growing season in the oat–lupin mixture. Although the negative relationship between Δ height and SE could not undermine the overall positive relationship between Δ height and the NE, it indicates that early in the growing season, higher growth of crops in the oat–lupin mixture contributed to a positive net biodiversity effect also via the selection effect.

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, i.e. 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 before that different crops in mixtures have differing strategies for acclimating to their light environments (Franco, King & Volder 2018). Photosynthetic capacity and leaf N are known to be closely linked, since more than 50% of total leaf N is allocated to the photosynthetic machinery (e.g. Rubisco) and other enzymes of the Calvin cycle (Evans & Clarke 2019). 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 are in line with earlier observations in this intercropping system (Engbersen *et al.* 2021a) 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 individuals (Ellsworth & Reich 1992); 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.

In the oat–camelina mixture, decreasing Δ leaf N contributed positively to the NE through the SE, indicating that mixtures had similar or lower leaf N than the monocultures. These observations are in line with other work where it was observed that species assemblages which were more similar in their traits contributed more strongly to the SE (Huang *et al.* 2020).

3.5. Conclusion

This study found evidence that the net biodiversity effect and both its components increased over time in both cereal–legume and cereal–non-legume mixtures. 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 contributing to complementarity. 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 net biodiversity effect, whereas in the oat–camelina mixture the positive net biodiversity effect 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. While further research is needed in this area, particularly the inclusion of a wider range of light-use related traits, the generated knowledge and improved understanding of mechanisms that contribute to light-driven yield advantages in intercropping systems can help optimize species combinations and thus make intercropping systems more successful.

Acknowledgments

General discussion

Key findings

This thesis was set out to investigate how temporal dynamics could enhance our understanding of the processes contributing to overyielding in mixed cropping systems. Intercropping is a promising concept for sustainable agriculture and has been found to improve and stabilize crop yields (Brooker *et al.* 2015). In intercropping, concepts such as spatial complementarity (Hauggaard-Nielsen, Ambus & Jensen 2001) and the inclusion of legumes (Bedoussac *et al.* 2015) have been identified to contribute to sustainable crop production. Moreover, relay intercropping systems use temporal differentiation between early and late sown crops to limit direct competition and extend the growing season (Lithourgidis *et al.* 2011). The results from this thesis demonstrate that temporal dynamics of nutrient capture and light-use are further mechanisms with a potential for exploitation in sustainable crop production. Understanding how temporally dynamic processes differ in crop mixtures consisting of different species combinations can help creating mixtures with temporal complementarity.

In the first part, I observed that the diversity-productivity relationship is context-dependent, as I observed overyielding at the temperate site in Switzerland but not at the dry site in Spain. The many reasons that could have led to differing diversity-productivity relationships at the two sites are discussed in the first chapter but are generally focused on climatic and soil differences between the two sites and highlight that diversity-productivity relationships are strongly context-dependent. Further studies analyzing the same intercrop combinations found further evidence for a strong context-dependence in soil microbial communities (Stefan *et al.* 2021) and weed communities (Stefan, Engbersen & Schoeb 2021), supporting the notion that biodiversity-productivity relationships are very complex (Loreau & Hector 2001).

I further observed that different underlying processes were responsible for seed yield increases from monocultures to mixtures and for seed yield increases from 2- to 4-species mixtures. Increasing diversity from monocultures to mixtures includes a change from intra- to inter-specific competition, thus individuals in a mixture are surrounded not only by individuals of their own species but also by individuals of a different species. As plants from the same species compete more strongly with each other than with plants from a different species, due to reduced niche overlap (Adler et al. 2018), it becomes obvious that when going from monoculture to mixture

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the plants will benefit from being surrounded by heterospecific neighbors. However, when increasing diversity from 2- to 4-species mixtures the proportion of inter- and intra-specific competition remains the same as in the 2-species mixture, and only the amount of neighbors from different functional groups increased from one to two in the 2- vs. the 4-species mixture. Being from different functional groups reduces the niche overlap between species and it has been suggested by earlier work that functional diversity is more important to increase ecosystem functioning than species richness *per se* (Kahmen et al. 2006).

Following up, I analyzed the temporal dynamics of nutrient capture, biomass accumulation and light-use associated traits in two mixtures that have proven to work well during the first years' experiment and overyielded even in the dry and more difficult growing-conditions at the Spanish site. Cereal–legume mixtures, represented by the species oat and lupin, are known to be effective combinations in intercropping due to complementary N uptake strategies and phosphorous facilitation (Bedoussac *et al.* 2015). Mixtures of cereals and Brassicaceae, represented by oat and camelina, are less common but cultivating Brassicaceae species is nevertheless promising as they could help meet the growing demand for vegetable oil and they show agronomic potential for biofuel production (Vollmann & Eynck 2015).

The two mixtures showed different pathways that led to overyielding. The oat–lupin mixture was characterized by a slow early establishment of the legume, and in combination with the legumes' ability to capture its nitrogen from fixing atmospheric N_2, the intercropped cereal showed a high rate of nitrogen capture. However, the high rate of N uptake for the cereal ceased after mid-growing season, which was probably due to competition for light. The analysis of light-use associated traits indicated that photosynthetic capacity, rather than photosynthetic efficiency, was a key process in the oat–lupin mixture. As photosynthetic capacity is tightly linked to leaf nitrogen contents, it suggests that complementary use of nitrogen played a key role in overyielding of this mixture. Although oat experienced more shading when intercropped with lupin compared to when grown in monoculture, it was still able to accumulate similar biomass as when grown in monoculture, which I suggest was due to a better availability of nitrogen in the mixture and that thus an higher photosynthetic capacity of the oat could offset the increased shading in the oat–lupin mixture.

In the oat–camelina mixture, oat accumulated nitrogen and phosphorous earlier than camelina, indicating early temporal partitioning of nutrient uptake that translated into earlier growth of oat. Later in the growing season, this positive belowground effect developed into aboveground competition since the earlier growth of oat seemed to have inhibited the growth of camelina due to shading. Contrary to the oat–lupin mixture, nitrogen did not seem to be a decisive factor in this system and this goes hand in hand with the result that increased photosynthetic efficiency – instead of capacity – was linked to yield advantages in this mixture.

These results show that understanding the temporal dynamics of the processes that lead to overyielding is essential in mixed cropping systems. For instance, knowing that oat has gained a substantial benefit in the oat–camelina mixture from strong early establishment and has later on limited growth of the neighboring camelina could be essential information for cultivating this mixture. By delaying the sowing of oat for a few weeks, camelina can be given a chance for early establishment and sufficient access to light, which might allow it to remain competitive against the oat and create yield advantages for both species in this mixture.

Limitations - Generalization of results

While the work presented in the first chapter of this thesis aimed to produce generalized results about the relationship between plant traits and the two additive components of the net biodiversity effect, the results suggest that no such general relationships exist. Studies from grassland systems suggest that overyielding is not *per se* a result of higher diversity but limited to certain environmental conditions or particular species combinations (Hooper & Dukes 2004). This again supports the thesis of biodiversity-productivity relationships being highly context-dependent and can not easily be generalized across differing environments. In this thesis, the discrepancy between the absence of overyielding at the Spanish site in the first chapter but a presence of overyielding in the experiment of the second and third chapter, which also took place in Spain, suggests that overyielding may be also limited to particular species combinations rather than only environmental conditions. Moreover, the further work presented in this thesis clearly showed that processes leading to yield advantages were different in mixtures consisting of mixing partners from different functional groups. Thus, to derive general relationships between plant traits and biodiversity effects, experimental intercropping systems should probably be limited to consist of always the

same two (for 2-species mixtures) or four (for 4-species mixtures) different functional groups but with differing species per functional group. This would allow to detect complementary or facilitative processes that are characteristic for specific functional group combinations and pin these positive interactions to a specific set of traits. Once these positive interactions are understood and pinned down, it would allow to optimize intercropping system by choosing species or cultivars that show ideal trait characteristics and then test these combinations in different environmental contexts. However, all of these differences and inconsistencies suggest that intercropping systems that function well in one situation are not necessarily transferable to other situations and therefore, much further research is needed to create locally adapted successful cropping combinations.

Limitations - Applicability of results to real farming systems

Oat, lupin and camelina are crops of agricultural interest, as laid out earlier in this thesis, and the machinery to sow, harvest and separate grains of most common crop species exist and are available to farmers (Bedoussac *et al.* 2015). In Switzerland, grain legumes are becoming more popular for animal feed, due to the aim of reducing the import of foreign feed in organic farming, particularly soy bean (FiBL 2021). As grain legumes cultivated as monoculture are plagued by weeds and diseases, cultivating grain legumes in mixed cultures with cereals is advised. Oat–lupin mixtures are promising for animal feed production, as protein contents in lupin are nearly as high as in soy bean (FiBL 2020). To further suppress weed growth, camelina is sometimes added to grain legume–cereal mixtures, which further enhances yield but also poses additional problems for grain separation (FiBL 2017). Thus, the choice of crop species used in this thesis is of current interest, at least in Switzerland, and addresses current incentives to improve the performance of crop mixtures, particularly mixtures with grain legumes.

Besides the choice of mixing partners, the spatial distribution of individuals in intercropping systems remains debated. For instance, it remains unclear whether it would be more efficient to completely mix species in an intercrop or to keep them in rows, which is often the case due to practical constraints for mechanical sowing and harvesting (Galanopoulou, Lithourgidis & Dordas 2019). In this thesis, the row distance of approx. 10 cm is not uncommon, as the standard inter-row distance for wheat in monocultures is approx. 10-16 cm (Drangmeister 2011). However, optimal spatial distributions in monocultures are not necessarily transferable to intercropping

systems and a recent study has shown that inter- and intra-row distancing can significantly influence crop yields (Stefan, Engbersen & Schoeb unpublished). For instance, the study demonstrated that yields of camelina are almost non-existent when oat is grown in 5 cm distance but significantly increase at 10 cm distance. The observation of high early exudation of organic acids in camelina individuals presented in the second chapter, could potentially explain the inhibiting effect of camelina on neighbors that are grown too close. Combining these insights can have important implications for understanding the underlying processes that define crop yields at different spatial configurations and further research is needed to investigate how plant-plant interactions change at varying spatial distribution.

Outlook and research needs

An ideal intercrop should display yield advantages, which can be achieved when the intercrops show complementarity and thus can exploit the full temporal and spatial dimensions of resource use (Stomph *et al.* 2020). However, one species is often a weaker competitor, leading to asymmetrical competition which can strongly limit the efficiency of the whole intercropping system. While the choice of species and management practices can alleviate asymmetrical competition to some point, breeding specifically for intercropping may be a better alternative in the long run (Annicchiarico *et al.* 2019). Breeding for intercropping should be based on an ecological framework that selects for patterns of complementary resource use between the component species. However, the understanding of the exact underlying processes that lead to complementarity in mixed cropping systems remains limited (Mason *et al.* 2020) and depends of the identity of the mixed species and environmental contexts. Thus, to achieve successful intercrops, it must first be understood how crop species' resource exploitation of above- and belowground space changes when they are grown in mixture compared to when they are grown in monoculture. Here, temporal or spatial complementarity are key processes that enable crop species to more fully exploit available resources and knowledge gaps reside largely on the side of temporal dynamics (Schofield *et al.* 2018). The thesis presented here has addressed some existing knowledge gaps but many more remain.

A major barrier for temporal studies is the sheer amount of work and space required to process and fit frequent destructive harvests. The development of non-destructive proxy measures

is therefore a key to make these types of experiment more feasible; time-, space- and cost-wise. For instance, non-destructive estimates of leaf nitrogen could be done by using a chlorophyll meter (Dunn, Singh & Goad 2018) and root architectural systems can be mapped nowadays using spectroscopy-based methods (Streit *et al.* 2019). Thus, I suggest that future work focuses on developing non-destructive ways to measure plant traits that are indicative of resource use.

Although this thesis has examined temporal dynamics of root exudates, I have only examined a small selection of organic acids, and organic acids are only one group within a large variety of compounds that can be released from plant root systems. Other studies have demonstrated that root exudates can also strongly modulate changes in the microbial community growing in the rhizosphere (Aira *et al.* 2010), be a key process for kin recognition that shapes plant responses to neighbors (Schofield 2020), negatively affect neighboring plants (Cheng & Cheng 2015), avoid pest attacks (Raza *et al.* 2015), and that composition and quantity of root exudates changes with plant development (Chaparro *et al.* 2013). The work presented in Chapter 2 of this study could be extended by including a wider range of root exudates or even examining temporal changes in microbial communities of the rhizosphere. As root-microbe-soil nutrient interactions are complex and this research is still in its infancy (Zhang, Vivanco & Shen 2017), many pathways for future research are possible to enhance our understanding of the fundamental links between soil, plant and microbial dynamics in mixed cropping systems.

Belowground root growth was not addressed in this study, even though root exploitation of the soil profile has been demonstrated to be highly variable when species are grown in mixture compared to when they are grown in monoculture (Hauggaard-Nielsen, Ambus & Jensen 2001; Streit, Meinen & Rauber 2019). For instance, significant differences in root biomass among eight genotypes of faba bean were observed when grown in mixture with a cereal, indicating a potential for selecting optimal cultivars to complement the root growth of a component crop (Streit, Meinen & Rauber 2019). Based on these insights, spatiotemporal dynamics of root growth among mixture components – and maybe even among cultivars – are key to limiting belowground competition for nutrients and enabling a full exploitation of the entire soil profile which will contribute to productivity and stability of the mixtures.

Last but not least, this thesis attempted to link specific processes to the complementarity and selection effects, which are the mechanisms explaining overyielding. Thanks to the additive

partitioning method, the presence of complementarity and selection effects can easily be measured (Loreau & Hector 2001). Similarly, resource partitioning on a spatial and – to a lesser extent – on a temporal gradient as well as facilitation have been demonstrated in many studies. But detecting resource partitioning or facilitation in a system does not explicitly tie them to the consequence of overyielding (Isbell *et al.* 2018; Barry *et al.* 2019). I therefore suggest that future studies investigating resource partitioning or facilitation in mixed cropping system should always link these processes with an increased performance of mixtures relative to monocultures. Otherwise, it remains unknown whether the detected resource partitioning or facilitation really is an underlying process that drives overyielding.

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Appendix 1

Figure A1: *Cross-reference of 8 samples for N and C leaf content analysis on LECO (LECO CHN628C elemental analyzer [Leco Co., St. Joseph, USA]) and MS (PDZ Europa 20-20 isotope ratio mass spectrometer linked to a PDZ Europa ANCA-GSL elemental analyzer [Sercon Ltd., Cheshire, UK]). Samples were analyzed on either device, depending on the available leaf dry mass. Samples with less than 100 mg (505 samples) available leaf dry mass were measured on MS and samples with more than 100 mg (400 samples) available leaf dry mass were measured on LECO.*
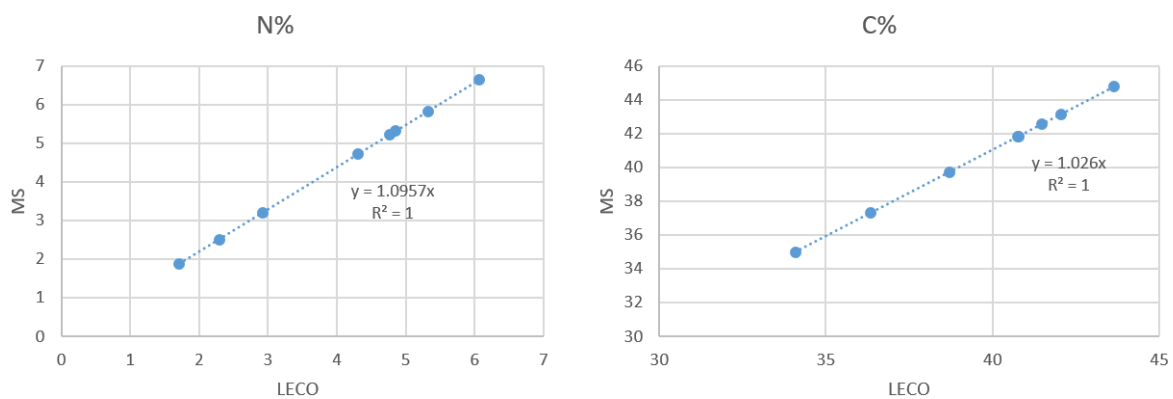
Figure A2: *A priori structural model for the relationship of variables analyzed with the structural equation model. The a priori model related environmental factors to the differences of plant traits and these to between mixtures and monocultures and these to selection and complementarity effects.*
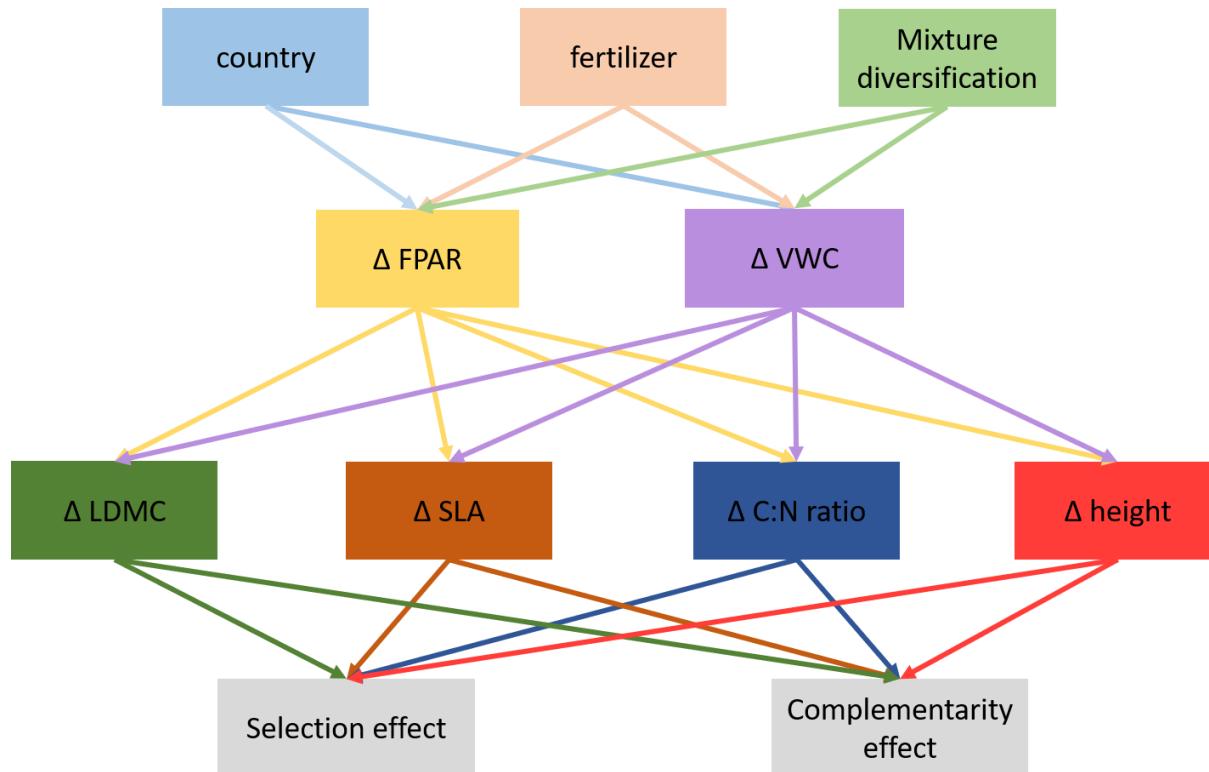
Figure A3: *ΔYield (A) and Δplant height (B) of the eight different species (Ave = Avena sativa, Cam = Camelina sativa, Cor = Coriandrum sativum, Len = Lens culinaris, Lin = Linum usitatissimum, Lup = Lupinus angustifolius, Qui = Chenopodium quinoa, Tri = Triticum aestivum) separated by country. Δ are calculated as value in mixture – mean value in monoculture. To make mixtures comparable with monocultures, we multiplied the value in mixture with the number of species. The red dotted line indicates 0. ΔValues above 0 indicate higher values in mixtures compared with monocultures and Δvalues below 0 indicate lower values in mixtures compared with monocultures.*
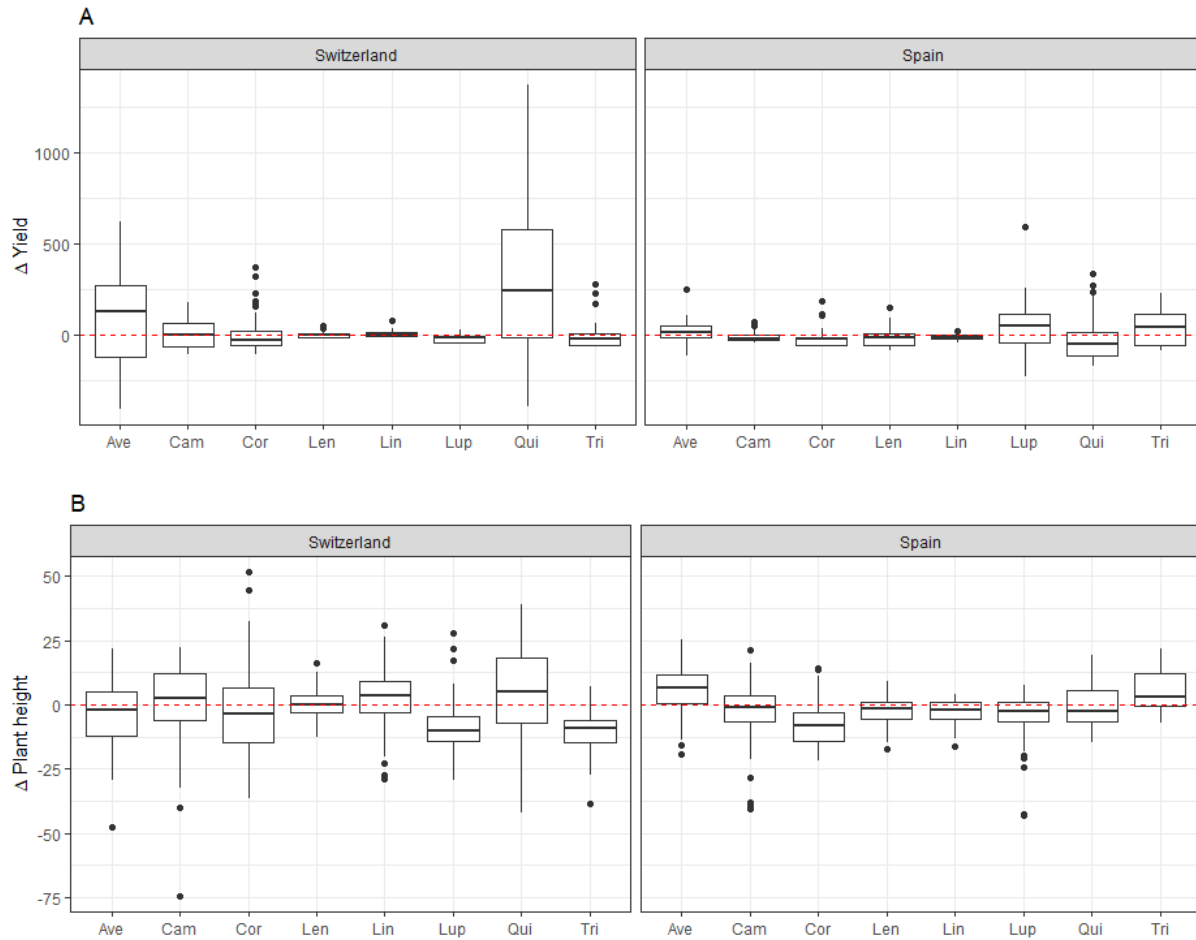
Figure A4: Model predictions for treatment effects on environmental factors (VWC, FPAR), plant traits (SLA, LDMC, plant height, leaf N, C:N ratio) and biodiversity effect components (CE, SE).
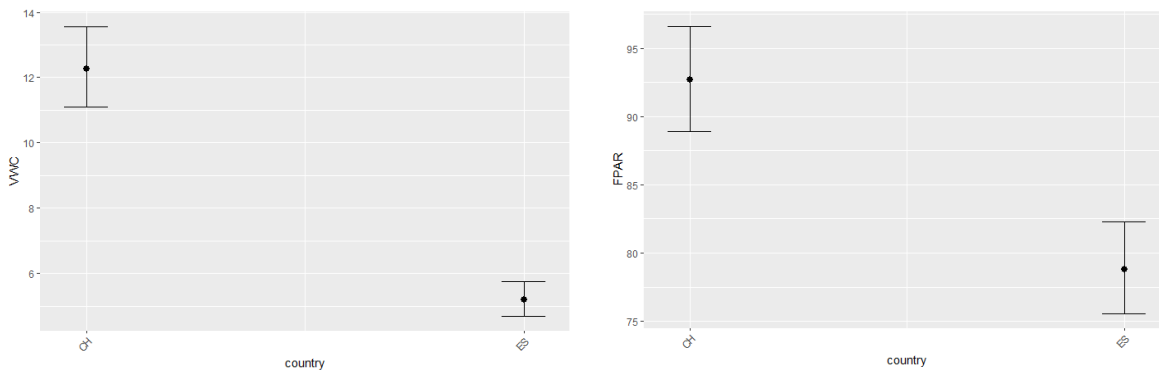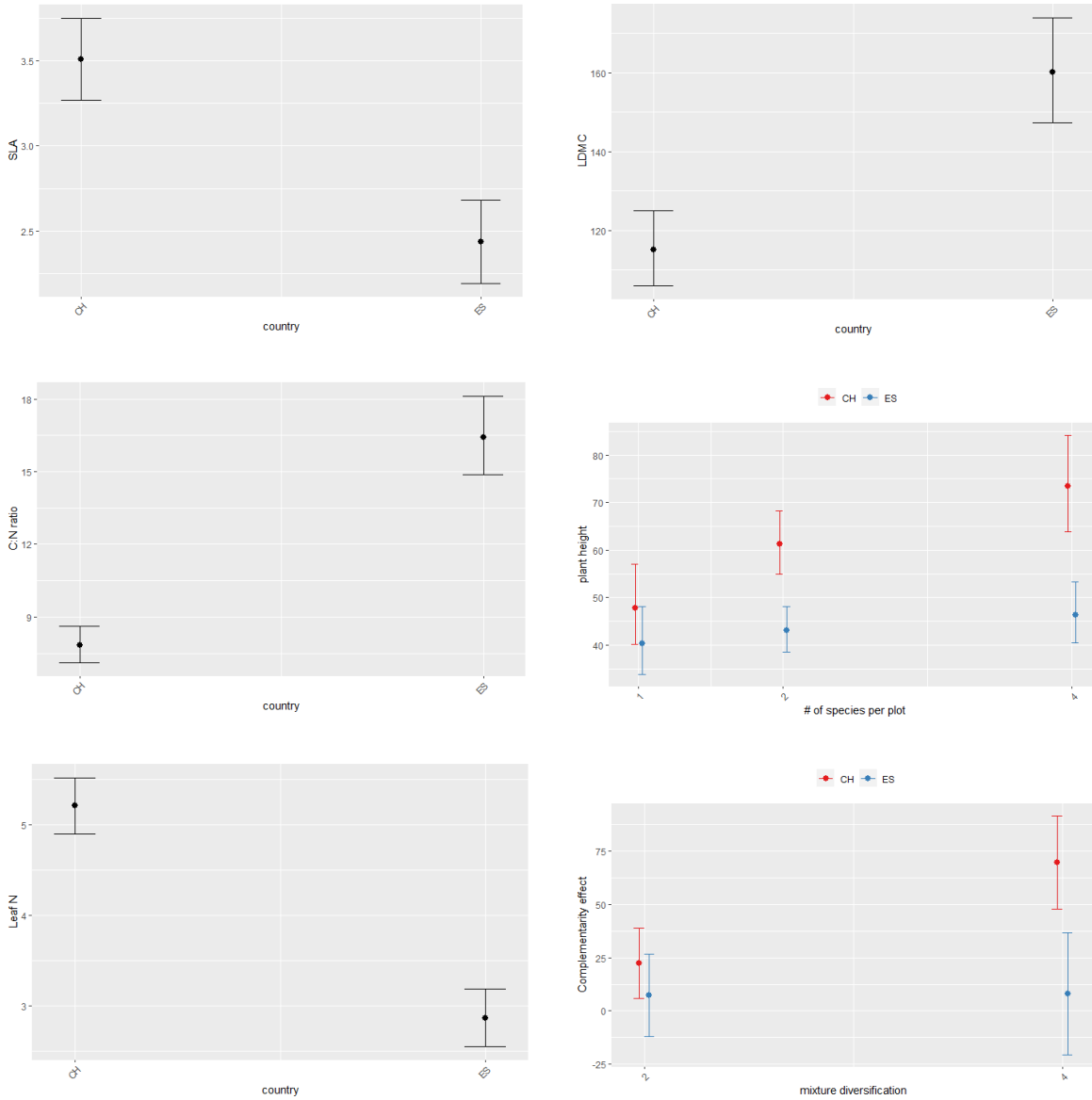
Figure A5: *Contribution of biodiversity effect components (SE, CE) to Δyield in %.*
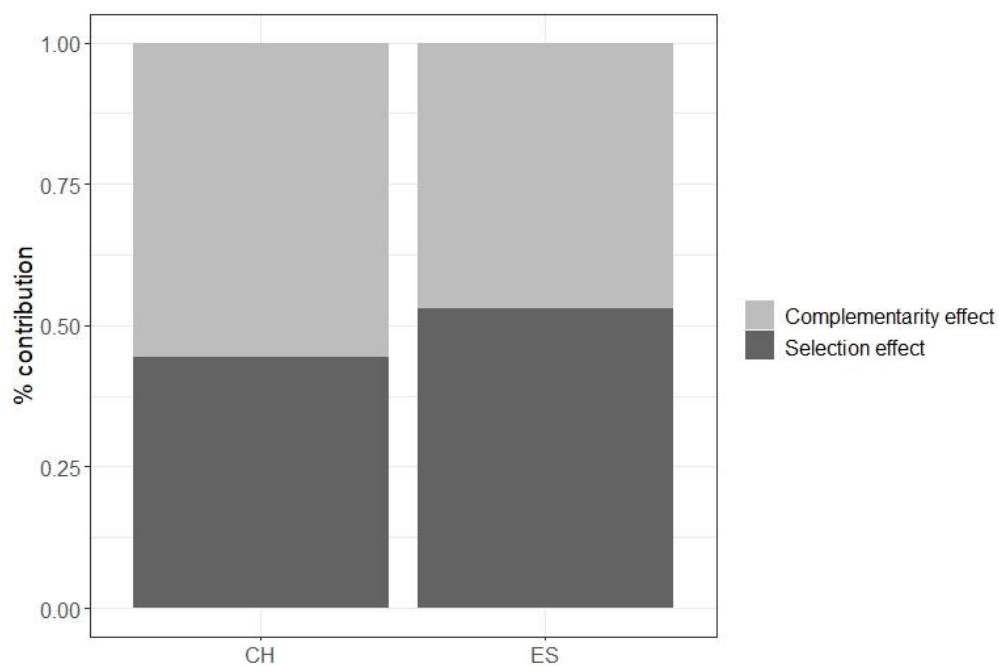
Table A1: *List of crop species phylogenetic groups, cultivar, seed supplier and their sowing densities.*

Species	Phylogenetic group	Cultivar	Supplier	Sowing density (seeds / m²)
Triticum aestivum	Cereal	Fiorina	DSP, Delley	400
Avena sativa	Cereal	Canyon	Sativa Rheinau	400
Lens culinaris	Legume	Anicia	Agroscope, Reckenholz	160
Lupinus angustifolius	Legume	Boregine	Aspenhof, Wilchingen	160
Camelina sativa	Superrosid	n.a.	Zollinger Samen, Les Evouettes	592
Linum usitatissimum	Superrosid	Lirina	Sativa Rheinau	592
Chenopodium quinoa	Superasterid	n.a.	Artha Samen, Münsingen	240
Coriandrum sativum	Superasterid	Indian	Zollinger Samen, Les Evouettes	240

Table A2: *Crop growth duration in mean days (± SD) from sowing to harvest for both countries and all eight species.*

Species	Days after sowing	
	Switzerland	Spain
Triticum aestivum	106 ± 9.9	146 ± 1.3
Avena sativa	120 ± 6.2	146 ± 6.2
Lens culinaris	128 ± 16.2	146 ± 1
Lupinus angustifolius	126 ± 15.7	134 ± 1.7
Camelina sativa	98 ± 1	153 ± 4.4
Linum usitatissimum	136 ± 3.1	155 ± 3.1
Chenopodium quinoa	142 ± 7.8	187 ± 6.4
Coriandrum sativum	123 ± 10.7	152 ± 5.5

Table A3: *Results of mixed effects ANOVA testing effects of the different treatments (country, fertilizer, legume, diversity (monocultures vs. mixtures) and mixture diversification (2- vs. 4-species mixtures) on community-level yield. SS: Sum of squares, MS: mean of squares, numDF: degrees of freedom of term, denDF: degrees of freedom of error term, F-value: variance ratio, P: error probability. P-values in bold are significant at α = 0.05 (* P < 0.05, ** P < 0.01, *** P < 0.001). n = 314.*

	SS	MS	numDF	denDF	F-value	P
country	4.691	4.691	1	24.98	12.64	**0.002****
fertilizer	1.192	1.192	1	23.75	3.212	0.086
legume	0.201	0.201	1	43.24	0.542	0.466
diversity	1.524	1.524	1	42.67	4.107	**0.049***
mixture diversification	1.933	1.933	1	43.78	5.209	**0.027***
country × fertilizer	0.384	0.384	1	23.65	1.035	0.319
country × legume	16.99	16.99	1	257.1	45.77	**8.91E-1*****
country × diversity	15.01	15.01	1	79.23	40.45	**1.20E-08*****
country × mixture diversification	24.95	24.95	1	246.2	67.22	**1.34E-14*****
fertilizer × legume	0.356	0.356	1	254.9	0.958	0.329
fertilizer × diversity	0.059	0.059	1	76.7	0.158	0.692

fertilizer × mixture diversification	0.014	0.014	1	244.7	0.037	0.849
country × fertilizer × legume	1.518	1.518	1	253.9	4.089	**0.044***
country × fertilizer × diversity	0.488	0.488	1	77.59	1.315	0.255
country × fertilizer × mixture diversification	0.997	0.997	1	245.5	2.685	0.103

Table A4: *Tukey post-hoc test of linear mixed effects model testing effects of the different treatments on community-level yield (results in Table A 1.1). P-values in bold are significant at α = 0.05 (* P < 0.05, ** P < 0.01, *** P < 0.001). CH = Switzerland, ES = Spain, mix = mixture, mono = monoculture, 2 = 2-species mixture, 4 = 4-species mixture. Only significant interactions are shown for the threeway-interaction country x fertilized x legume.*

contrast	estimate	SE	df	t.ratio	p.value
~ country x diversity					
CH,mix-ES,mix	1.02	0.11	33.7	9.21	**<.0001*** **
CH,mix-CH,mono	1.59	0.30	54	5.25	**<.0001*** **
CH,mix-ES,mono	0.82	0.3	52.5	2.72	0.042
ES,mix-CH,mono	0.57	0.3	52.8	1.9	0.243
ES,mix-ES,mono	-0.2	0.31	55.9	-0.65	0.915
CH,mono-ES,mono	-0.77	0.17	103.4	-4.49	**0.0001*** *
~ country x mixture diversification					
2,mix,CH-4,mix,CH	-1.36	0.27	53.5	-5.12	**0.0001*** *
2,mix,CH-mono,CH	0.91	0.29	53.1	3.09	**0.036***
2,mix,CH-2,mix,ES	0.25	0.12	31.4	2.2	0.268
2,mix,CH-4,mix,ES	0.42	0.27	58.6	1.56	0.63
2,mix,CH-mono,ES	0.14	0.3	54	0.47	0.997
4,mix,CH-mono,CH	2.27	0.36	55.5	6.25	**<.0001*** **
4,mix,CH-2,mix,ES	1.61	0.27	59.2	5.96	**<.0001*** **

4,mix,CH-4,mix,ES	1.78	0.17	99	10.45	**<.0001***
4,mix,CH-mono,ES	1.5	0.36	52.9	4.18	**0.002**
mono,CH-2,mix,ES	-0.65	0.3	54.1	-2.21	0.25
mono,CH-4,mix,ES	-0.49	0.36	52.6	-1.36	0.751
mono,CH-mono,ES	-0.77	0.17	103.4	-4.49	**0.0003***
2,mix,ES-4,mix,ES	0.17	0.26	49.9	0.63	0.988
2,mix,ES-mono,ES	-0.12	0.3	55.3	-0.39	0.999
4,mix,ES-mono,ES	-0.28	0.36	56.2	-0.77	0.971

~ country x legume

CH,no - ES,no	1.158	0.132	154.3	8.761	**<.0001***
CH,no - CH,yes	1.274	0.256	54.2	4.985	**<.0001***
CH,no - ES,yes	0.364	0.259	56.2	1.406	0.501
ES,no - CH,yes	0.116	0.261	57.9	0.445	0.9704
ES,no - ES,yes	-0.794	0.259	56.4	-3.068	0.0169
CH,yes - ES,yes	-0.91	0.131	145.3	-6.954	**<.0001***

~ country x fertilizer x legume

CH,no,no - ES,no,no	1.4574	0.186	150	7.851	**<.0001**
CH,no,no - ES,yes,no	0.8878	0.186	155.7	4.768	**0.0001**
CH,no,no - CH,no,yes	1.4651	0.284	80.1	5.16	**<.0001**
ES,no,no - CH,yes,no	-1.429	0.186	149.1	-7.676	**<.0001**
ES,no,no - ES,no,yes	-1.1006	0.291	85.5	-3.785	**0.0066**
CH,yes,no - CH,no,yes	1.4367	0.289	83	4.973	**0.0001**
CH,yes,no - CH,yes,yes	1.0837	0.283	79.2	3.831	**0.0059**
CH,no,yes - ES,no,yes	-1.1083	0.185	150.6	-5.979	**<.0001**
CH,no,yes - ES,yes,yes	-1.0656	0.184	142.6	-5.796	**<.0001**
ES,no,yes - CH,yes,yes	0.7552	0.185	145.3	4.073	**0.0019**
CH,yes,yes - ES,yes,yes	-0.7125	0.185	137.9	-3.862	**0.0042**

Table A5: *Mean ± SE for community-weighted means of environmental factors and plant traits in Switzerland (CH) and Spain (ES), with and without application of fertilizer in monocultures (mono), 2-species mixtures (2-sp-mix) and 4-species mixtures (4-sp-mix). Measurements were taken at the time of flowering.*

country	fertilized	diversity	VWC [%]	FPAR [%]	SLA [cm^2 g^{-1}]	LDMC [mg g^{-1}]	plant height [cm]	leaf N [%]	C:N ratio
CH	no	mono	13.3 ± 1.56	90.8 ± 3.46	3.69 ± 0.35	116.9 ± 7.63	54 ± 6.24	5.3 ± 0.22	7.8 ± 0.39
		2-sp-mix	11.9 ± 0.71	93.4 ± 1.07	3.65 ± 0.15	121.8 ± 3.56	55.3 ± 3.04	5.24 ± 0.09	7.96 ± 0.18
		4-sp-mix	11.5 ± 0.7	95.2 ± 0.99	3.98 ± 0.19	116.2 ± 3.37	52.9 ± 2.29	5.17 ± 0.13	8.05 ± 0.23
	yes	mono	14.3 ± 1.16	93.2 ± 2.35	3.44 ± 0.23	119.2 ± 7.76	55.5 ± 5.6	4.93 ± 0.28	8.8 ± 0.9
		2-sp-mix	13.6 ± 0.92	95.6 ± 0.79	3.7 ± 0.17	116.4 ± 3.49	53.6 ± 2.44	5.1 ± 0.11	8.19 ± 0.28
		4-sp-mix	12.7 ± 0.96	95.7 ± 0.74	3.48 ± 0.12	120.6 ± 2.68	55.1 ± 2.35	4.73 ± 0.25	9.56 ± 0.77
ES	no	mono	5.45 ± 0.74	74.7 ± 4.86	2.49 ± 0.17	166 ± 14.6	37.4 ± 3.84	3.08 ± 0.34	17.7 ± 2.64
		2-sp-mix	5.87 ± 0.49	81.1 ± 2.55	2.63 ± 0.07	163.9 ± 5.84	37.1 ± 1.57	2.9 ± 0.14	17.3 ± 1.11
		4-sp-mix	5.91 ± 0.69	80.2 ± 3.1	2.59 ± 0.07	173.7 ± 5.46	35.2 ± 0.99	2.93 ± 0.08	17.6 ± 0.81
	yes	mono	6.15 ± 0.78	83.4 ± 3.37	2.78 ± 0.2	143.5 ± 8.36	44 ± 3.74	2.68 ± 0.36	19 ± 2.19
		2-sp-mix	5.28 ± 0.3	84.5 ± 1.81	2.87 ± 0.12	157.6 ± 6.37	44.3 ± 1.61	2.92 ± 0.16	17.3 ± 1.08
		4-sp-mix	5.85 ± 0.56	80.3 ± 2.56	2.57 ± 0.07	163.1 ± 5.69	44.4 ± 1.2	2.69 ± 0.07	18.6 ± 0.67

Table A6: *Results of mixed effects ANOVA testing effects of the different treatments (country, fertilizer, legume, diversity (monocultures vs. mixtures) and mixture diversification (2- vs. 4-species mixtures) on environmental variables (VWC, FPAR), plant traits (SLA, LDMC, plant height, leaf N, C:N ratio) and biodiversity effects (complementarity & selection effects). Df: degrees of freedom, denDF: denominator degrees of freedom, F.inc: incremental Wald F statistics, P: error probability. P-values in bold are significant at $\alpha = 0.05$ (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). n = 314.*

	Df	denDF	F.inc	P	
VWC					
(intercept)	1	295	125.9	**<2e-16**	***

country	1	295	4.385	**0.04**	*
fertilizer	1	295	0.041	0.84	
diversity	1	295	0.029	0.86	
mix. diversification	1	295	0.003	0.96	
country x fertilizer	1	295	0.034	0.85	
country x diversity	1	295	0.008	0.93	
country x mix. diversification	1	295	0.014	0.91	
fertilizer x diversity	1	295	0.001	0.98	
fertilizer x mix. diversification	1	295	0	0.99	
country x fertilizer x diversity	1	295	0.014	0.91	
country x fertilizer x mix. diversification	1	295	0.004	0.95	

FPAR

(intercept)	1	296	104.6	**2.20E-16**	***
country	1	296	10.8	**0.001**	**
fertilizer	1	296	0.5	0.481	
diversity	1	296	0.4	0.526	
mix. diversification	1	296	0.04	0.839	
country x fertilizer	1	296	0.02	0.891	
country x diversity	1	296	0.09	0.76	
country x mix. diversification	1	296	0.15	0.695	
fertilizer x diversity	1	296	0.11	0.745	
fertilizer x mix. diversification	1	296	0.09	0.771	
country x fertilizer x diversity	1	296	0.04	0.842	
country x fertilizer x mix. diversification	1	296	0	0.964	

SLA

(intercept)	1	296	302	**2.20E-16**	***
country	1	296	10	**0.002**	**
fertilizer	1	296	0.23	0.634	

diversity	1	296	0.28	0.595	
mix. diversification	1	296	0.26	0.611	
country x fertilizer	1	296	0.23	0.633	
country x diversity	1	296	0.2	0.657	
country x mix. diversification	1	296	0.61	0.436	
fertilizer x diversity	1	296	0.06	0.805	
fertilizer x mix. diversification	1	296	0.11	0.737	
country x fertilizer x diversity	1	296	0.03	0.861	
country x fertilizer x mix. diversification	1	296	0	0.959	

LDMC

(intercept)	1	296	289.4	**2.20E-16**	***
country	1	296	8.6	**0.004**	**
fertilizer	1	296	0.3	0.586	
diversity	1	296	0.2	0.652	
mix. diversification	1	296	0.18	0.675	
country x fertilizer	1	296	0.08	0.784	
country x diversity	1	296	0.05	0.822	
country x mix. diversification	1	296	0.22	0.643	
fertilizer x diversity	1	296	0	0.956	
fertilizer x mix. diversification	1	296	0	0.984	
country x fertilizer x diversity	1	296	0.04	0.841	
country x fertilizer x mix. diversification	1	296	0.04	0.847	

Plant height

(intercept)	1	68.7	10950	**2.20E-16**	***
country	1	50.8	76.1	**1.12E-11**	***
fertilizer	1	49.9	0.8	0.373	
diversity	1	49.1	5.2	**0.027**	*
mix. diversification	1	53.7	2.5	0.118	

country x fertilizer	1	50.5	3.2	0.08	.
country x diversity	1	54.6	12.8	**0.001**	***
country x mix. diversification	1	34	6.4	**0.016**	*
fertilizer x diversity	1	52.4	1	0.317	
fertilizer x mix. diversification	1	33.4	0	0.905	
country x fertilizer x diversity	1	53.4	0.9	0.358	
country x fertilizer x mix. diversification	1	34.4	0.4	0.517	

Leaf N

(intercept)	1	296	119.4	**<2e-16**	***
country	1	296	1.096	0.296	
fertilizer	1	296	0.014	0.9068	
diversity	1	296	0.004	0.9519	
mix. diversification	1	296	0.001	0.9817	
country x fertilizer	1	296	0	0.9873	
country x diversity	1	296	0	0.9846	
country x mix. diversification	1	296	0	0.9915	
fertilizer x diversity	1	296	0.001	0.977	
fertilizer x mix. diversification	1	296	0.001	0.9808	
country x fertilizer x diversity	1	296	0	0.9846	
country x fertilizer x mix. diversification	1	296	0	0.9923	

C:N ratio

(intercept)	1	296	274.9	**2.20E-16**	***
country	1	296	37.68	**0**	***
fertilizer	1	296	0.29	0.591	
diversity	1	296	0.36	0.548	
mix. diversification	1	296	0.12	0.731	
country x fertilizer	1	296	0.25	0.616	
country x diversity	1	296	0.01	0.906	

country x mix. diversification	1	296	0.02	0.89	
fertilizer x diversity	1	296	0.01	0.946	
fertilizer x mix. diversification	1	296	0	0.998	
country x fertilizer x diversity	1	296	0	0.992	
country x fertilizer x mix. diversification	1	296	0.15	0.698	
Complementarity effects					
(intercept)	1	22.4	15.53	**0.001**	***
country	1	24.7	8.18	**0.009**	**
fertilizer	1	19.8	0.85	0.367	
mix. diversification	1	35.9	8.67	**0.006**	**
country x fertilizer	1	22.1	0.25	0.623	
country x mix. diversification	1	197.1	6.73	**0.01**	*
fertilizer x mix. diversification	1	165.3	1.95	0.164	
country x fertilizer x mix. diversification	1	170.4	0.03	0.865	
Selection effects					
(intercept)	1	31.3	24.14	**2.69E-05**	***
country	1	24	1.06	0.315	
fertilizer	1	15.2	2.36	0.145	
mix. diversification	1	35.8	1.99	0.167	
country x fertilizer	1	18	0.97	0.338	
country x mix. diversification	1	192.5	0	0.979	
fertilizer x mix. diversification	1	164.6	0.2	0.654	
country x fertilizer x mix. diversification	1	168.1	0.08	0.784	

Table A7: *Mean values ± standard errors of the differences of plant traits, yield and environmental factors between community-level means in mixtures compared to community-level means in monocultures. Δ values were calculated according to Eqn. 1.1-1.3 (see*

methods). Negative Δ values indicate higher community-level means in monocultures compared with mixtures.

country	CH				ES			
fertilized	no		yes		no		yes	
species nr.	2	4	2	4	2	4	2	4
ΔFPAR	2.5 ± 1.34	4.44 ± 1.11	2.39 ± 0.67	3.18 ± 0.75	6.52 ± 2.2	5.35 ± 3.06	1.03 ± 1.95	-3.24 ± 2.41
ΔVWC	-1.4 ± 0.82	-1.75 ± 0.68	-0.71 ± 0.77	-1.65 ± 0.89	0.38 ± 0.49	0.39 ± 0.74	-0.84 ± 0.36	-0.11 ± 0.56
ΔLDMC	0.55 ± 0.29	-0.12 ± 0.35	0.67 ± 0.4	0.95 ± 0.33	0.18 ± 0.34	1.65 ± 0.98	1.55 ± 0.47	2.53 ± 0.52
ΔSLA	-0.26 ± 0.11	0.02 ± 0.15	0.02 ± 0.12	-0.17 ± 0.11	-0.02 ± 0.05	-0.29 ± 0.08	-0.04 ± 0.09	-0.41 ± 0.08
ΔC:N ratio	-0.15 ± 0.14	0.07 ± 0.24	-0.17 ± 0.36	1.24 ± 0.9	-2.16 ± 0.83	-2.09 ± 0.86	-2.31 ± 0.63	-3.23 ± 0.64
Δheight	1.76 ± 2.12	1.59 ± 2.8	1.43 ± 1.83	3.03 ± 2.26	4.34 ± 1.16	7.36 ± 1.61	4.4 ± 0.86	8.21 ± 1.53
CE	1.82 ± 1.28	6.2 ± 1.22	2.95 ± 1.12	6.71 ± 2.17	-0.29 ± 0.59	0.08 ± 0.98	1.81 ± 0.97	0.5 ± 0.49
SE	3.78 ± 1.12	5.34 ± 1.64	2.8 ± 1.2	4.97 ± 2.34	1.27 ± 0.36	1.5 ± 0.58	0.11 ± 0.34	0.97 ± 0.29
Δyield	55 ± 18.03	115.4 ± 16.8	57.5 ± 12.3	116.8 ± 23.4	9.9 ± 5.94	15.9 ± 9.47	19.2 ± 9.72	14.7 ± 5.19

Appendix 2

Table A1: Parameters indicating the fit of logistic nls models. AvLu: oat with lupin, AvCa: oat with camelina, AvAv: oat monoculture, LuAv: lupin with oat, LuLu: lupin monoculture, CaAv: camelina with oat, CaCa: camelina monoculture. RSS: Residual sum of squares, R^2: ratio of residual sum of squares (RSS) to total sum of squares (TSS). #it: Number of iterations to reach model convergence. Df: degrees of freedom.

	RSS	R^2	# it.	Achieved convergence tolerance	Residual Std. Error	Df
Biomass						
AvLu	5.84	0.74	0	8.36E-06	0.3384	51
AvCa	7.79	0.82	0	4.26E-06	0.3948	50
LuAv	1026.36	0.7	0	5.69E-06	4.486	51
CaAv	6.29	0.29	6	8.44E-06	0.3548	50
AvAv	3.79	0.81	0	8.78E-06	0.2724	51
LuLu	324.18	0.79	4	8.94E-06	2.521	51
CaCa	3.88	0.54	0	4.58E-06	0.2786	50
Av	1860.5	0.59	1	4.84E-06	6.1	50
Lu	43182.8	0.8	2	6.99E-06	29.1	51
Ca	4949.76	0.43	5	8.46E-06	9.852	51
Phosphorous						
AvLu	207.02	0.33	4	3.62E-06	2.627	30
AvCa	89.93	0.72	1	3.76E-06	1.731	30
LuAv	7064.28	0.72	14	9.58E-06	16.18	27
CaAv	75.82	0.24	9	8.7E-06	1.646	28
AvAv	280.63	0.45	4	7.92E-06	3.111	29
LuLu	3339.77	0.68	3	6.68E-06	10.55	30
CaCa	56.19	0.34	7	8.12E-06	1.369	30
Av	9409.82	0.39	0	5.3E-06	17.71	30
Lu	230553.4	0.83	4	7.36E-06	92.41	27
Ca	19746.9	0.2	0	5.6E-06	31.42	20
Nitrogen						
AvLu	2879.2	0.47	3	6.3E-06	8.089	44
AvCa	1656.65	0.71	5	6.75E-06	6.136	44
LuAv	1394605	0.64	5	7.12E-06	176	45
CaAv	6349.42	0.24	14	8.37E-06	12.44	41
AvAv	686.38	0.72	1	8.79E-06	3.905	45
LuLu	444691.9	0.73	10	8.83E-06	99.41	45

CaCa	1005.7	0.6	15	9.54E-06	4.953	41
Av	234828.9	0.62	1	4.57E-06	77.6	39
Lu	83597206	0.69	3	9.57E-06	1446	40
Ca	1125044	0.41	1	7.74E-06	197	29

Table A2: *Mean $\delta^{15}N$ values $\pm$ SEM of leaves of lupin that are fully dependent upon N_2 fixation and sampled at the same harvest week as the field plants (β-values). n = 3 per week.*

| harvest week	β
1	1.33 ± 0.06
2	0.8 ± 0.26
3	0.82 ± 0.26
4	-0.22 ± 0.04
5	-0.43 ± 0.1
6	-0.79 ± 0.39
7	-0.54 ± 0.08
8	-0.71 ± 0.15
9	-0.84 ± 0.1
10	-0.84 ± 0.11
11	-0.94 ± 0.06
12	-0.94 ± 0.1
13	-1.1 ± 0.24
14	-1.23 ± 0.1
15	-1.38 ± 0.22
16	-1.55 ± 0.15

Table A3: *I_{max} values for each species in mixture, monoculture or as isolated single plant. I_{max} is the maximum instantaneous biomass accumulation (g day^{-1}) and nutrient uptake rate (mg day^{-1}) which emerges at the time t_{max}.*

	Biomass	Phosphorous	Nitrogen
Oat : Lupin	0.15	1.51	8.89
Oat : Camelina	0.25	2.11	3.61
Oat mono	0.18	2.35	3.56
Oat single	2.91	6.59	42.67
Lupin : Oat	4.38	19.78	175.76
Lupin mono	2.61	10.73	103.85

Lupin single	69.04	284.7	2684.4
Camelina : Oat	0.25	0.77	5.65
Camelina mono	0.13	1.03	3.33
Camelina single	3.99	34.99	116.62

Table A4: *Results of linear mixed effects ANOVA testing the effects of diversity (mixture, monoculture, isolated singles) and harvest week on %Ndfa. Random term was replicate. SS: Sum of squares, MS: mean of squares, Df: degrees of freedom, F-value: variance ratio. P-values in bold are significant at α = 0.05 (* P < 0.05, ** P < 0.01, *** P < 0.001).*

	Df	SS	MS	F-value	P
diversity	2	15591.9	7796	47.267	**<0.001*****
harvest week	11	3032.7	275.7	1.672	0.1
diversity × harvest week	22	2198.4	99.9	0.606	0.905
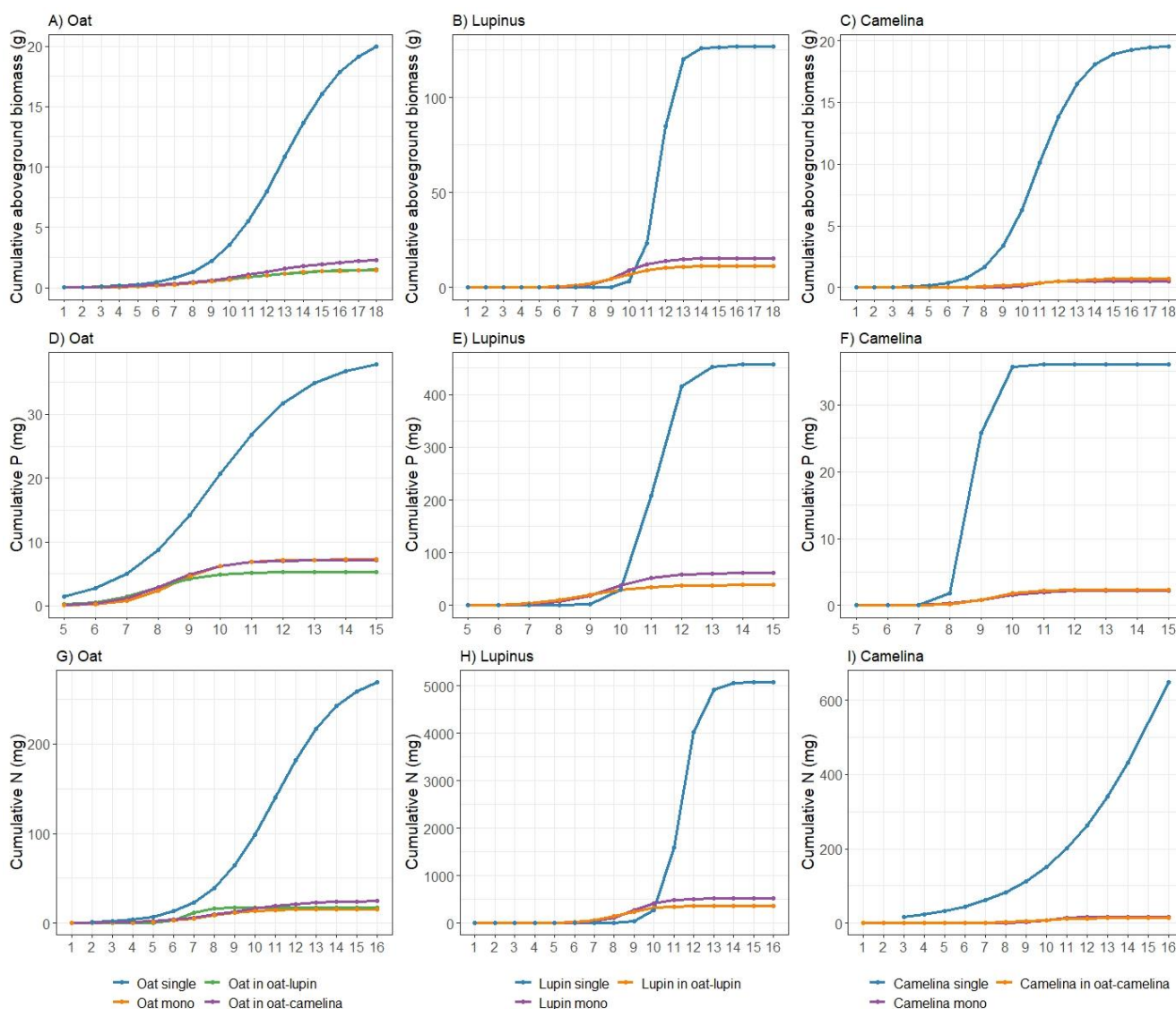
Figure A1: *Trajectories of cumulative aboveground biomass (A-C), P (D-F) and N (G-I) uptake of oat with lupin (A, D, G: green), oat with camelina (A, D, G: purple), lupin (B, E, H) and camelina (C, F, I) when grown in mixture (purple, green), in monoculture (orange) and as isolated singles (blule). Same data as in Fig. 2.2 but with addition of isolated singles, which were removed from Fig. 2.2 to aid comparisons between the other treatments. Curves are derived from Eqn. 2.1. Note different y-axis scales.*
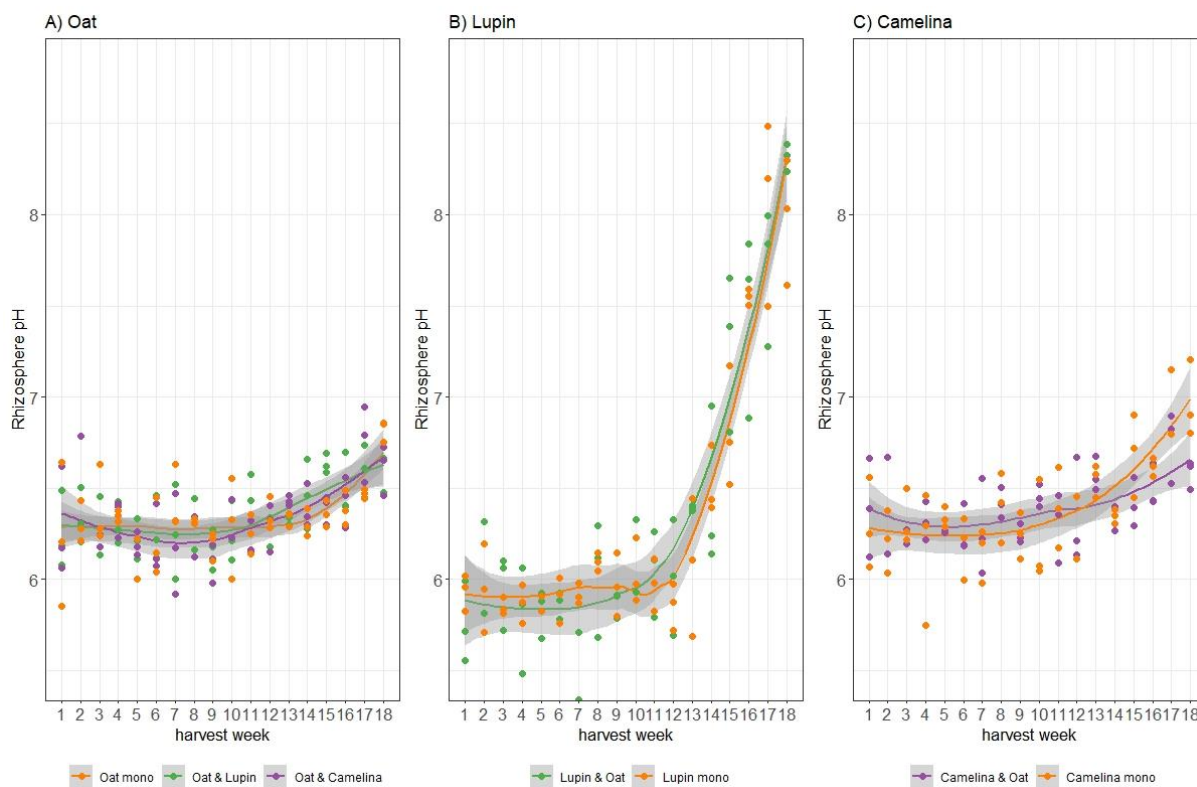
Figure A2: *Rhizosphere pH measured in rhizosphere soil immersed in 0.2 mM CaCl₂ solution for oat (A), lupin (B) and camelina (C) in monoculture (orange) or mixture (green, purple). Shading refers to standard errors computed using a t-based approximation.*

Appendix 3

Table A1: *Pearson's coefficients of correlation for community weighted means of all light-use related traits.*

	Δ height	Δ diameter	Δ ΦPS$_{II}$	Δ leaf N	Δ FPAR
Δ height	1	0.46	-0.1	0.08	0.16
Δ diameter	0.46	1	-0.06	0.26	-0.02
Δ ΦPS$_{II}$	-0.1	-0.06	1	0.1	0.12
Δ leaf N	0.08	0.26	0.1	1	0.22
Δ FPAR	0.16	-0.02	0.12	0.22	1

Table A2: *ANOVA table showing results of linear model testing the effects of block, mixture composition (mix., i.e. oat-lupin vs. oat-camelina) and harvest week (HW, i.e. harvest weeks 17-18) on the net biodiversity effect and its two additive components, the complementarity and selection effect. Biodiversity effects (NE, CE, SE) are based on total grain yield to represent the reproductive part (harvest weeks 17-18). SumSq: Sum of squares, F-value: variance ratio, P: error probability. P-values in bold are significant at α = 0.05 (* P < 0.05, ** P < 0.01, *** P < 0.001).*

net biodiversity effect				
	Df	SumSq	MS	F-value	P
block	2	12.54	6.27	0.551	0.599
mix.	1	7.93	7.93	0.697	0.431
HW	1	71.78	71.78	6.313	**0.04***
Residuals	7	79.58	11.37		

complementarity effect				
	Df	SumSq	MS	F-value	P
block	2	14.08	7.04	0.641	0.555
mix.	1	3.89	3.89	0.354	0.57
HW	1	79.82	79.82	7.27	**0.031***
Residuals	7	76.86	10.98		

selection effect				
	Df	SumSq	MS	F-value	P
block	2	2.31	1.16	1.582	0.271
mix.	1	10.01	10.01	13.705	**0.008****
HW	1	3.63	3.63	4.967	0.061
Residuals	7	5.12	0.73		