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Growth and nitrogen economy of cereal-legume sole- and intercrops, and their effects on weed suppression

JAMES AJAL

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James Ajal
Faculty of Natural Resources and Agricultural Sciences
Department of Crop Production Ecology
Uppsala

SWEDISH UNIVERSITY
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Cover: Artistic illustration of a pea-barley intercrop with *Chenopodium album* and *Galeopsis spp.* weeds species growing below the canopy
(photo: J Ajal)

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Abstract

Crop production currently faces the dilemma of using methods that increase crop yield but with adverse environmental effects, or attaining lower yields with less environmental impacts. Applying ecological principles has shown potential in finding a middle-ground of maintaining crop yield with less inputs. In this thesis, I evaluated the role of intra– and interspecific functional diversity of cereal–legume intercrops in increasing nitrogen accumulation efficiency, competition against weeds, and improving the productivity of the mixture compared to sole crops. Pea-barley and faba bean-wheat species combinations were grown in the field in Uppsala, Sweden and Taastrup, Denmark as sole crops and two-species mixtures. In addition, a pot experiment with various combinations of faba bean, wheat, and a common weed was set up under semi-controlled conditions in Sweden. Both crops and weeds were evaluated for nitrogen economy, biomass accumulation, and grain yields. Using trait space analysis, I demonstrated that intercropping, cultivar identity, and environmental differences influence trait space through phenotypic plasticity. Furthermore, intercropping facilitated more N acquisition in the cereals compared to when grown in sole crop. For crop-weed interaction, pea-barley intercrop reduced weed biomass compared to the sole-cropped pea, and the weed species' competitiveness and dominance was influenced by nitrogen availability in the soil. Based on the results presented in this thesis, there is need to pay more attention to the functional traits of species components when designing mixtures. The weed species composition should be taken into account before nitrogen addition, otherwise, the additional nitrogen may favour mostly weed growth, at the expense of the crops. This study is timely considering the current emphasis on crop production methods that use ecological principles to address challenges in arable farming.

Keywords: cereal-legume, intercropping, nitrogen use efficiency, functional traits, weed suppression, crop mixtures, trait space, hypervolumes

Author's address: James Ajal, SLU, Department of Crop Production Ecology, P.O. Box 7043, 750 07, Uppsala, Sweden.

Biomassaproduktion och kväveeffektivitet hos kombinationer av stråsäd och baljväxter odlade i rena och blandade bestånd, samt effekterna på ogräsminskning

Sammanfattning

Inom växtproduktionen står vi idag inför dilemmat att odlingmetoderna antingen är intensiva med höga skördar och stor miljöpåverkan eller, för att få en mindre miljöpåverkan, mindre intensiva men då också ger lägre skördar. Att använda ekologiska principer för växtproduktion kan ha en god potential som medelväg för att bibehålla höga skördenivåer med mindre externa resurser. I denna avhandling har jag utvärderat betydelsen av intra- och interspecifik funktionell diversitet i samodlingssystem med spannmål och baljväxter för att öka effektiviteten av kväveupptaget i grödan samt konkurrenskraften mot ogräs, och för att förbättra grödornas produktivitet jämfört med om de odlas var för sig. Kombinationer av korn-ärt och vete-åkerböna odlades i fältförsök i Uppsala, Sverige och Taastrup, Danmark i renbestånd och i blandningar. Utöver detta genomfördes ett krukförsök med olika kombinationer av åkerböna, vete och ett vanligt ogräs under semikontrollerade odlingsförhållanden i Sverige. Både grödor och ogräs analyserades utifrån kvävehushållning, biomassatillväxt och kärnskörd. Genom en statistisk metod baserad på nischvolymer för en rad olika växtegenskaper visade jag att samodling, sortidentitet och miljöskillnader påverkar nischvolymerna genom fenotypisk plasticitet. Dessutom ledde samodlingen till att mer kväve togs upp av spannmålen jämfört med när de odlades i renbestånd. För interaktionen mellan gröda och ogräs minskade korn-ärt blandningen ogräsbiomassan jämfört med ärt i renbestånd och konkurrenskraften och dominansen av olika ogräsarter påverkades av kvävetillgången i marken. Baserad på resultaten i denna avhandling föreslår jag att vara mer uppmärksam på de funktionella egenskaperna hos de artkomponenter som man använder i blandningar. Sammansättningen av ogräsarter i fält bör tas i beaktning innan kvävegödsling genomförs, annars kan det extra kvävet gynna ogrästillväxt på bekostnad av grödan. Denna studie kommer lägligt med tanke på tonvikt som just nu läggs på metoder som använder ekologiska principer för att möta utmaningar inom växtodlingen.

Nyckelord: spannmål-baljväxt, samodling, kväveeffektivitet, funktionella egenskaper, ogräsminskning, artblandning, nischvoly

Dedication

To dr. ir. Nicole Smit, and in memory of dr. ir. Paul Robert Speijer and Prof. Anne van den Ban

You have not lived today until you have done something for someone who can never repay you.

John Bunyan

Contents

List of publications 9

1. Introduction 11
   - 1.1 Intercropping for sustainable intensification 11
     - 1.1.1 Maintaining crop yields using less inputs 13
     - 1.1.2 Resilience and yield stability in intercrops 13
   - 1.2 Functional trait diversity in intercrops 15
     - 1.2.1 Inter and intraspecific trait diversity 15
     - 1.2.2 Trait-based approach for understanding intercrop performance 17
   - 1.3 Nitrogen use efficiency in intercrops 18
     - 1.3.1 Different aspects of nitrogen use efficiency 18
     - 1.3.2 The role of intercrops in increasing nitrogen use efficiency 18
   - 1.4 Crop–weed interaction in intercrops 19
     - 1.4.1 Crop yield loss associated with weeds 19
     - 1.4.2 Weed management from an ecological perspective 20
     - 1.4.3 Weed suppression in intercrops 20

2. Aim 23

3. Material and Methods 25
   - 3.1 Plant material and experimental design 25
     - 3.1.1 Plant material 25
     - 3.1.2 Field study 27
     - 3.1.3 Pot study 27
   - 3.2 Measurements and biomass sampling 32
   - 3.3 Nutrient analysis and calculations 33
     - 3.3.1 Nitrogen availability to the crop and weeds 33
     - 3.3.2 Nitrogen accumulation efficiency and its components 34
     - 3.3.3 Analysis of other macronutrients 35
   - 3.4 Trait space analysis 35
   - 3.5 Statistical analysis 35

4. Results and Discussion 37
4.1 The role of intercropping in shaping plant interaction and functional trait spaces 38
4.2 Species-specific N economy in sole crops and intercrops of cereals and legumes. Are biomass and grain yield affected? 40
4.3 Crop-weed interaction, weed suppression and N accumulation in crops and weeds 45

5. Conclusions and recommendations 49

6. Future prospects 51

References 53

Popular science summary 61

Populärvetenskaplig sammanfattning 65

Acknowledgments 69

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. **Ajal, J.**, Jäck, O., Vico, G., & Weih, M. (2021). Functional trait space in cereals and legumes grown in pure and mixed cultures is influenced more by cultivar identity than crop mixing. *Perspectives in Plant Ecology, Evolution and Systematics* **50**, 125612. https://doi.org/10.1016/j.ppees.2021.125612

II. **Ajal, J.**, Kiær, L P., Pakeman, R J., Scherber, C. & Weih, M. Intercropping and environmental differences drive phenotypic plasticity and changes in functional trait space (Submitted manuscript)

III. Jäck, O., **Ajal, J.** & Weih, M. (2021). Altered nitrogen availability in pea–barley sole- and intercrops changes dominance of two nitrophilic weed species. *Agronomy*, *11*(4), 679. https://doi.org/10.3390/agronomy11040679

IV. **Ajal, J**. & Weih, M. More nitrogen accumulation in mixtures of faba bean and wheat through optimized cultivar choice (Submitted manuscript)

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The contribution of James Ajal to the papers included in this thesis was as follows:

I. Participated in the conceptualization of the study, conducted the fieldwork, analyzed the data, interpreted the results in collaboration with the co-authors, led the writing of the manuscript with substantial input from the co-authors.

II. Conceptualization of the study together with the co-authors, conducted the fieldwork in the Swedish site, analyzed the data, interpreted the results, led the writing of the manuscript with substantial input from the co-authors.

III. Participated in establishing the experiment, conducted fieldwork, and reviewed and edited the manuscript in collaboration with the co-authors.

IV. Conceptualization of the study together with the co-author, conducted the experiment, analyzed and interpreted the data together with the co-author, led the writing of the manuscript with substantial input from the co-author.

1. Introduction

1.1 Intercropping for sustainable intensification

Mixing species has been practiced for a long time, as a method of intensive production (Gliessman, 1985). So what makes such an old practice relevant today?

Over the years, there has been tremendous progress in crop production technologies that have increased production efficiency and boosted yields of major crops such as rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.*)*. But the yield increase is associated with unintended environmental impacts that result from large amounts of chemical pesticides and inorganic fertilizers (Foley et al., 2011; Tilman, 2020). The consequences of these environmental threats include a reduced population of non-target beneficial organisms (e.g., pollinator insects), development of pesticide resistance, and invasion of non-indigenous species of weeds, pests/diseases, among others (Theoharides and Dukes, 2007). Alternative practices that ensure a self-sustaining system without compromising the environment and biodiversity are recommended (Bommarco et al., 2013).

Species mixtures (or intercrops) have shown the potential to counteract the over-dependence on synthetic inputs, and thus mitigate the challenges with sole cropping. Intercropping is the cultivation of two or more crop species on the same field (Vandermeer, 1989). Depending on the intended goal, species can be mixed within rows (row intercropping), in strips with several rows of each species (strip intercropping), or separated in time due to the difference in sowing time of each species (relay intercropping). The introduced diversity in intercrops ensures the species are separated in time and space, and thus facilitate temporal and spatial niche differentiation and complementarity depending on the method used (Stomph et al., 2020). In this thesis, different cultivars of cereal and legume species were sown as sole crops and as two-species intercrops mixed within rows. The potential benefits from intercrops range from more efficient resource use (Jensen et al., 2020; Li et al., 2020), reduced diseases and pest pressure (Boudreau, 2013; Brooker et al., 2015), and increased and stable yields that are resilient to extreme weather (Lin, 2011; Raseduzzaman and Jensen, 2017; Weih et al., 2021). With the current extreme climate events, the role of crop diversification (e.g., intercropping) in increasing yield stability and resilience, and the reduction of synthetic fertilizer use has been a focal point of discussion at both research and policy levels (Jacobs et al., 2019).

In the tropical regions, intercropping is a major land management system, characterized by high instances of subsistence agriculture with intensive labor and low inputs (Brooker et al., 2015; Martin and Sauerborn, 2013). In the European perspective, intercropping is mainly practiced under organic systems where the use of external synthetic inputs is prohibited, and particularly, cereal-legume intercrops are used (Li et al., 2020; Voisin et al., 2014). The cereals wheat, barley (*Hordeum vulgare* L.*)*, oats (*Avena sativa* L.*)*, and maize (*Zea mays*) are commonly used while the grain legumes pea (*Pisum sativum* L.*)*, faba bean (*Vicia faba* L.*)*, and soybean *(Glycine max* L.*)* are the most common crops used in intercropping (Watson et al., 2017). Several studies focus on barley-pea and wheat-faba bean intercrops (Hauggaard-Nielsen et al., 2009; Hauggaard-Nielsen and Jensen, 2001; Weih et al., 2021). This thesis work builds on these preceding works done on barley-pea and wheat-faba bean species combinations. Particularly, several cultivars have been developed, some with different functional trait values, which makes finding suitable species/cultivar combinations for designing highly productive intercrops a challenge (Haug et al., 2021). Therefore, there is need for more research to explore the opportunity of using dedicated species/cultivar mixtures to maximize intercrop performance, either through field agronomic studies or selective breeding for legume-based intercropping (Annicchiarico et al., 2019) .

1.1.1 Maintaining crop yields using less inputs

The ability for intercrops to achieve maintained or even greater crop yields using less external inputs (e.g., herbicide, pesticides, and fertilizers) compared to growing the same crops in pure cultures is not only beneficial to the environment but also economically sound. Although the benefits associated with intercrops have been linked to low input systems, a review by Stomph et al. (2020) highlighted why this argument is not completely exclusive. This suggests a need to rethink the current application of intercropping to low input subsistence or organic agriculture and explore its potential to conventional high input systems, typical in temperate regions. For example, in legume-supported intercrops, grain yields of up to 16–29% per hectare can be attained using 19–36% lower amount of fertilizer compared to the sole crops (Li et al., 2020). In another global study, intercropping cereals with legumes reduced the need for fertilizer application by 26% (Jensen et al., 2020). The key explanation for the observed trend is the often higher resource use efficiency in intercrops compared to sole crops. Higher resource use efficiency can lead to higher crop productivity and also increase the fertilizer nitrogen (N) recovery, consequently reducing the amount of residual fertilizer N that may be prone to leaching from the soil (Yan et al., 2014).

1.1.2 Resilience and yield stability in intercrops

Attaining resilient and stable crop systems that can resist and/or rebound from disturbances is the focus of most diversity-related research. According to Holling (1973), resilience is a measure of how ecological systems resist and absorb change when faced with unpredicted perturbations, while stability is the ‘ability of a system to return to an equilibrium state after a temporary disturbance’. Although contested, grain legumes grown as sole crops are considered to have variable yields, hence less stable compared to the non-legumes (Cernay et al., 2015; Watson et al., 2017). However, published results are partly contradictory, not least due to different methodologies used: In a recent study, more stable grain yields have been recorded in cereal-legume intercrops compared to the corresponding sole crops only under more productive conditions (Weih et al., 2021), whilst another study concluded generally more stable yields in the intercrops grown under various conditions (Raseduzzaman and Jensen, 2017). One way intercrops achieve more stable yields can be through compensation (Raseduzzaman and Jensen, 2017), where a loss in one crop is offset by a higher yield in another. Another theory for the stability of more diverse plant communities is the insurance hypothesis, which suggests that in more diverse plant communities, there is a higher possibility of having individuals or species that easily adjust to different growth conditions and perform ecosystem functions than, for instance, a single species (Vogel et al., 2019; Wagg et al., 2017b). These properties place intercropping as a promising cropping practice for the future, especially where more regular occurrence of extreme weather patterns is expected (Toreti et al., 2019).

Box 1. Glossary

Intercropping is the cultivation of two or more crop species on the same field (Vandermeer, 1989).

Intraspecific diversity is the variation in plant characteristics or phenotypes of the same species (Mansion-Vaquié et al., 2019).

Interspecific diversity is the variation in plant characteristics or phenotypes of different species (Mansion-Vaquié et al., 2019).

Functional traits are morpho-physio-phenological plant characteristics that indirectly affect plant fitness and influence individual performance in terms of growth, reproduction and survival (Violle et al., 2007).

Phenotypic plasticity is the ability of a single genotype to express different phenotypes under different environmental conditions (Arnold et al., 2019; Bradshaw, 1965).

1.2 Functional trait diversity in intercrops

1.2.1 Inter and intraspecific trait diversity

Intra- and interspecific functional diversity is central in regulating the functioning of natural plant communities (Lee et al., 2016). In arable cropping systems, this property of natural systems can be mimicked through intercropping, where different species and/or cultivars are grown (Fig.1).

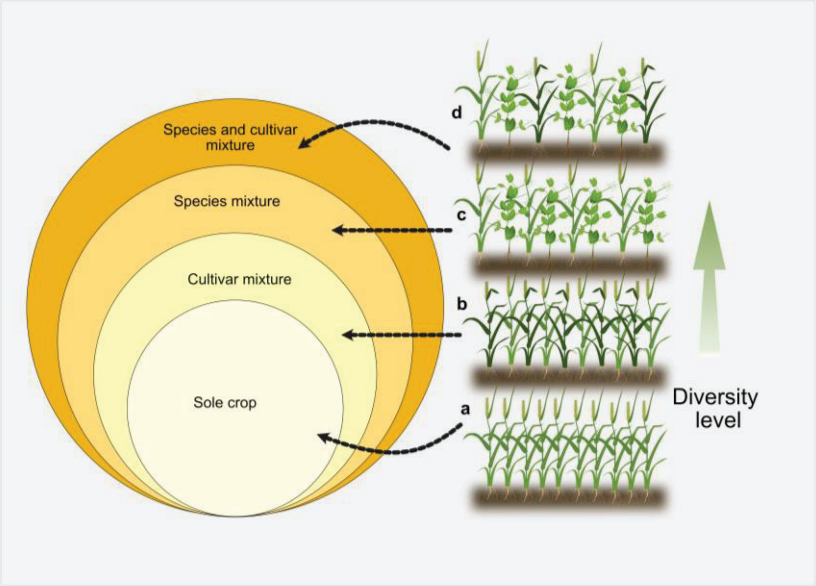


Figure 1. Illustration of hypothesized levels of crop diversity from the lowest (a) to highest (d) using pea and barley as model crops. The lowest level of diversity is represented by a sole cropped field of barley of the same cultivar (a). The crops are considered to have more uniform functional traits with low intraspecific variability. Intraspecific competition may be considered the highest here. Although sole cropped, different cultivars may have different trait values of the same trait and therefore show greater intraspecific trait variability than the sole cropped single cultivar (b). Because species mixtures may have components that differ in architecture, growth habits, etc. (c), mixtures have a greater potential to utilize common growth-limiting resources more efficiently, through complementarity, and may also benefit from facilitation, for example through N_2 fixation of N by pea (legume) to the barley. There is a greater variation in functional traits here. Major works of this thesis were done at this level. The highest level of diversity may be achieved through species and cultivar mixtures (d). The potential benefits may be similar to (c) above. Interspecific competition may be considered the lowest here. Illustration: James Ajal.

The balance between intra- and interspecific diversity is key in designing a successful intercrop given that it can determine whether complementarity or competition will dominate the plant-plant interactions.

Complementarity

Complementarity is attributed to two mechanisms; facilitation and niche differentiation (Loreau and Hector, 2001). Collectively, these mechanisms enhance plant growth through beneficial interactions when sharing common but limited resources (Brooker, 2006; Dong et al., 2018; Macek et al., 2016).

With facilitation, the interactions between two or more species result in the benefit of either one or all species in the interaction (Brooker et al., 2021). For intercropped species, N fixation by the legumes for use by non-legumes, allopathic effects by some cereals (e.g., barley and wheat) against weeds (Reiss et al., 2018), and phosphorus (P) uptake by mycorrhizal fungi (Wang et al., 2019) are common examples of facilitation. Niche differentiation involves the temporal or spatial partitioning of resources from the resource pool, and is associated with more efficient resource use in more diverse systems (Wagg et al., 2017a). For example, intercropped species that differ in their growth rate or maturity may induce a yield advantage in the intercrops than the same species in a sole crop (Dong et al., 2018). Furthermore, species with different rooting architecture and depth facilitate a more complete resource exploration from the soil profile (Guderle et al., 2018). Quantifying the individual contribution of facilitation and niche differentiation to the overall complementary effect is always a challenge and in most cases quantified as a combined effect, for example in Loreau and Hector (2001).

Competition

Plants are in continuous interaction with their neighbors, especially for the acquisition of growth-limiting resources. During competition, plants make demands for resources in excess of supply, which results in a net negative effect (Craine et al., 2013; Weaver and Clements, 1938). A yield advantage in intercrops occurs when interspecific competition is less than intraspecific competition. In nature, plants have evolved different strategies to minimize competitive stress and have a greater share of the limiting resources for growth (Went, 1973). For arable cropping systems where fields are highly managed, the farmer can, to a limited extent, manipulate intra- and interspecific competition through different spatial and temporal cropping designs. For example, growing functionally different crops in an intercrop, or separating the species in time and space to lower interspecific competition (Dong et al., 2018).

1.2.2 Trait-based approach for understanding intercrop performance

The use of the mean trait values is a common practice to quantify plant performance in many agronomic studies. This quantification is based on the assumption that intraspecific trait variation is too small to affect the outcome of the measured trait values (Violle and Jiang, 2009). Ideally, both intra- and interspecific trait variation should be accounted for. As a compromise, a multi-trait analysis can be considered. Many ecological theories related to trait-based approaches have been applied (Violle et al., 2007). Over the years, their application has expanded to agro-ecosystems, specifically aiming to connect the different ecological processes and functions to plant diversity (Bukovsky-Reyes et al., 2019). In arable cropping systems, trait-based approaches have been used in different contexts, for example in crop-weed competition (Pakeman et al., 2015). The advantage of focusing on functional traits is that it creates a clear link between biotic and abiotic factors, and plant responses, and vice versa (Schellberg and Pontes, 2012).

Crop species performance in intercrops, like many other plants in arable agricultural systems, are attributed to niche differentiation, complementarity, or competition (Martin and Isaac, 2015). Even though approaches exist for measuring some of the mentioned mechanisms, for example, complementary effects by Loreau and Hector (2001) in biodiversity experiments such as intercrops, the mechanistic link to functional traits remains unclear. Since plant traits can directly or indirectly affect species performance in the environment they are grown in, they can be useful in quantifying niches (Violle and Jiang, 2009). Therefore, the trait space approach, based on *n*-dimensional hypervolumes (Blonder et al., 2014; Blonder et al., 2018), can be a good indicator for species niches, which is central in intercropping studies, where two or more species share the same limited resources during their growth period. In this thesis, hypervolumes were defined for functional trait spaces via the differential functional traits of the cereals and legumes at different diversity levels, and were used to evaluate trait variability with species at different diversity levels. Furthermore, in the calculation of scaling exponents of the crops grown with and without weeds, stoichiometric niche volumes were used to assess the stoichiometric relations between N and phosphorus (P) *vs.* other nutrients, i.e., calcium (Ca), potassium (K), magnesium (Mg), and sulfur (S) (Ågren and Weih, 2020). Here, the dimensions and sides of the multidimensional shape, as well as the element concentrations of the nutrients, are considered.

1.3 Nitrogen use efficiency in intercrops

1.3.1 Different aspects of nitrogen use efficiency

Several methods have been proposed to evaluate N use efficiency in plants (Craswell and Godwin, 1984; Moll et al., 1982; Weih et al., 2011; Weih et al., 2018). A broad characterization of N use efficiency is based on the difference approach, balance approach, and ^{15}N tracer approach (Quan et al., 2021). No standard definition exists in the literature, but in most cases plant-based N use efficiency is composed of N uptake efficiency and N utilization efficiency. The approach by Weih et al. (2018) was used throughout this thesis to evaluate N accumulation efficiency (NAE) and its components in the different species grown in sole crops and intercrops. It takes into account the N use efficiency components mentioned above, but also accommodates the N stored in the grain, which accounts for the N carry over in annual crops. Therefore the N in the grain seed is incorporated in the calculation of N uptake efficiency (U_N). Soil N status is not directly accounted for in the analysis, but the mean plant N pool during the season and grain seed N indirectly accounts for this.

1.3.2 The role of intercrops in increasing nitrogen use efficiency

Many studies that focus on intercropping cite resource use efficiency as one of the benefits (Brooker et al., 2015; Fan et al., 2020; Gou et al., 2017). Especially for cereal-legume intercropping, where the atmospheric N fixed by the legumes can be utilized by the non-legumes, N use efficiency plays a central role in the productivity of the intercrop. Cereals are highly competitive crops compared to the legumes and, when grown as sole crops, the high intraspecific competition results in lower N accumulation than in the intercrop (Bedoussac and Justes, 2010). Since legumes can meet much of their N demand from fixed N, in an intercrop, a large proportion of soil N is then available to be utilized by the cereals (Hauggaard-Nielsen et al., 2003). Furthermore, the high N demand by the cereals in the intercrops is a precursor for increased symbiotic N fixation by the legumes, since there is a negative correlation between the N fixing ability of the legumes and the amount of available N (Duchene et al., 2017). High N (especially nitrate) availability often reduces the N fixation ability of the legumes during the major growth period until grain filling (Voisin et al., 2002). These biological processes and plant-plant interactions among cereals and legumes that enhance N use efficiency are fostered by complementarity and facilitation, thanks to the dissimilarity in plant traits of the cereals and legumes that enhance spatial resource partitioning.

1.4 Crop–weed interaction in intercrops

1.4.1 Crop yield loss associated with weeds

Weed infestation in crop fields is one of the most important biotic constraints lowering crop production globally. Global loss from weeds is currently estimated to be about 200 million metric tons of grain (Chauhan, 2020). Yield losses may vary depending on the field location (environmental conditions), weed species present, and crop species. With the growing human population – currently estimated to be about 7.8 billion (UNFPA, 2021), coupled with yield losses related to other biotic factors or deteriorating climate, meeting the global food requirement is threatened. Attempts to meet this food demand have been addressed with several weed control measures, some of which are effective but have other negative consequences for the environment.

Weeds compete with crops for growth-limiting resources such as light, water, nutrients, among others. In most cases, the competition is through direct uptake of the shared resources or may take other indirect forms such as parasitism (e.g., *Striga spp.*) or allopathy (e.g., *Cirsium arvense*). Therefore, control options should aim at reducing weed competitiveness against the crops through weed population management. The use of herbicides, mechanical weed control, and cultural methods have for long been employed. Specifically, herbicides have been regarded as highly effective but their negative effects on human and animal health, and on the environment, have initiated the adoption of an integrated approach (integrated weed management – IWM) that employs several but complementary control measures (Chauhan, 2020; Colbach et al., 2020). Specifically, intercropping is one of the important weed management options that is based on the principles of IWM (Weerarathne et al., 2017).

1.4.2 Weed management from an ecological perspective

The emergence of herbicide resistance has rendered many previously effective herbicides less effective and yet the rotation of herbicides or combining different herbicides only offers temporary solutions (Bagavathiannan and Davis, 2018). Besides, great emphasis is being placed on crop production methods that minimize the use of synthetic agrochemicals (e.g., herbicides), owing to their negative effects on the environment (Brooker et al., 2021). Ideally, mimicking nature, and allowing the natural ecosystem functions to prevail would offer a more sustainable solution (Malézieux, 2011). Certainly, it is an uphill task to achieve this mimicry in arable cropping systems where fields are intensively managed and disturbed. Interventions such as introducing diversity (e.g., species or cultivars mixtures) into the system, using weed competitive cultivars, reducing the weed seed bank in the soil, and crop rotations (Gage and Schwartz-Lazaro, 2019), point in the right direction and offer promising solutions. In addition, the competitive relations between crops and weeds can be manipulated by regulating plant population density and spatial arrangement (Avola et al., 2008), taking into account the ecology and biology of the weeds (Chauhan, 2020).

1.4.3 Weed suppression in intercrops

Different crops or cultivars differ in their competitiveness against weeds, but in general, the competitiveness of cereals against weeds surpasses that of many other arable crops (Kolb and Gallandt, 2012). This difference in competitiveness also implies that the associated yield losses (due to weeds) for these crops, when grown as sole crops, would be different, with the less competitive crops being more affected. Previous studies have shown that cereal–legume intercrops often are more competitive against weeds and result in less accumulated weed biomass compared to the component species, particularly the legume, grown as sole crop (Corre-Hellou et al., 2011; Stomph et al., 2020).

Light capture and nutrient (especially N) acquisition are two main resource-dependent processes that determine crop-weed competition without taking into account seedling density and emergence time (Swanton et al., 2017). Intercrops are often more efficient in acquiring light within the canopy, and thus have a better soil cover compared to the corresponding sole crops (Zhu et al., 2015). Similarly, a better soil N accumulation by the intercrop ensures that less N is available for uptake by the weed compared to the sole crops that constitute the intercrop (Corre-Hellou et al., 2011).

The performance of the intercrops may be associated with the difference in traits of respective components, which enable spatial and temporal sharing of niche spaces (for mixed and relay intercropping respectively) and facilitates more resource capture by the components in intercrop at the expense of the weed (Dong et al., 2018). While the crop functional traits are important in crop-weed interaction, understanding the weed characteristics is critical in determining the competitive relations between the crop and the weed. For example, Swanton et al. (2017) highlighted that differences in the weed root system, leaf area development, or plant height can determine the competitive ability of different weed species. In intercrops, where there may be different crop competitive relations than in the corresponding sole crops, N availability to the crop (and weed) may be altered. The individual characteristics of the weeds, in this case, can determine their responsiveness to N availability in terms of shoot and root growth (Blackshaw et al., 2003).

2. Aim

The overall aim of this thesis was to determine how the intra- and interspecific functional diversity in cereal–legume intercrops (facilitated by differential functional traits) influence intercrop performance, in terms of nitrogen accumulation efficiency (NAE), biomass and grain yield, and interaction with weeds as compared to the corresponding sole crops.

This aim was tested based on the following specific objectives;

i) Determine how functional trait space, based on different cereal and legume traits, is influenced when cultivars of different species are grown as sole crops or intercrops (papers I and II).

ii) Investigate how cereals and legumes grown as intercrops influence different NAE aspects compared to the sole crop, and the subsequent effect on biomass accumulation and grain yield (papers I, III, and IV).

iii) Examine the differential effects of cereal-legume intercropping and weed characteristics on crop biomass accumulation, weed N accumulation and suppression compared to the component species grown as sole crops (papers III and IV).

I used a combination of field and pot experiments to evaluate the aim and set of objectives (Fig. 2). Two-species combinations i.e., pea-barley and faba bean-wheat, were grown in the field as sole crops and intercrops, and selected cultivars of faba bean and wheat, grown with and without a common weed were further evaluated in a pot experiment.

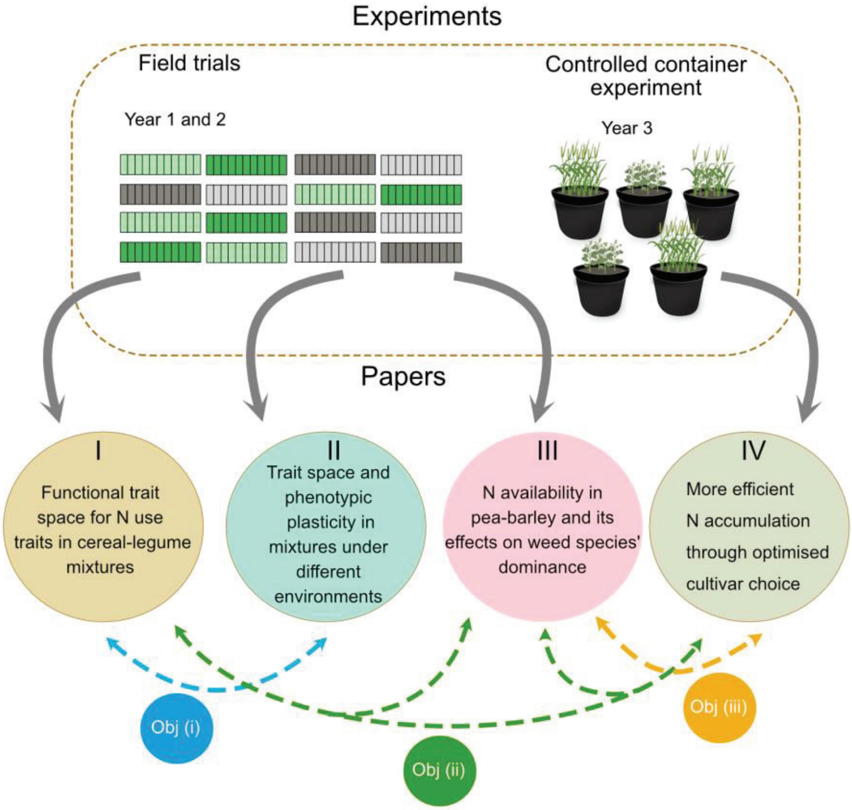


Figure 2. Conceptual diagram showing the relationships between the experiments conducted, and how the different papers and objectives (obj) that constitute this thesis are interrelated. The large colored circles represent the four different papers. The small blue, green and orange circles are the specific objectives of this thesis and the arrows show the connection of the objectives with the different papers. Illustration: James Ajal.

3. Material and Methods

This thesis was based on data from field experiments conducted in the spring seasons of 2017 and 2018 in Uppsala, Sweden (SE) – **papers I, II**, and **III**, and Copenhagen, Denmark (DK) – the partial data for **paper II**. In addition, a pot experiment under semi-controlled conditions was established in Uppsala, Sweden, in 2018 – **paper IV**.

3.1 Plant material and experimental design

3.1.1 Plant material

In this thesis, both crops and weeds were used as plant material. Two legume crops; pea and faba bean, and two cereal crops; spring barley and spring wheat, were grown as sole crops and as two-species intercrops of pea-barley and faba bean-wheat (Fig. 3). In the Swedish (Uppsala) field study, three cultivars of each cereal were used, and two cultivars of each legume, resulting in 12 species/cultivar combinations (for details, see paper I). For the purpose of comparability with the field study in Sweden, only species combinations with at least one common cultivar in the Danish (Taastrup) study were selected, except for a few cases (Appendix in paper II). The weed species used were those commonly found growing in fields with spring cereals in Sweden, e.g., lamb's quarters (*Chenopodium album)* and hemp-nettle (*Galeopsis spp).*

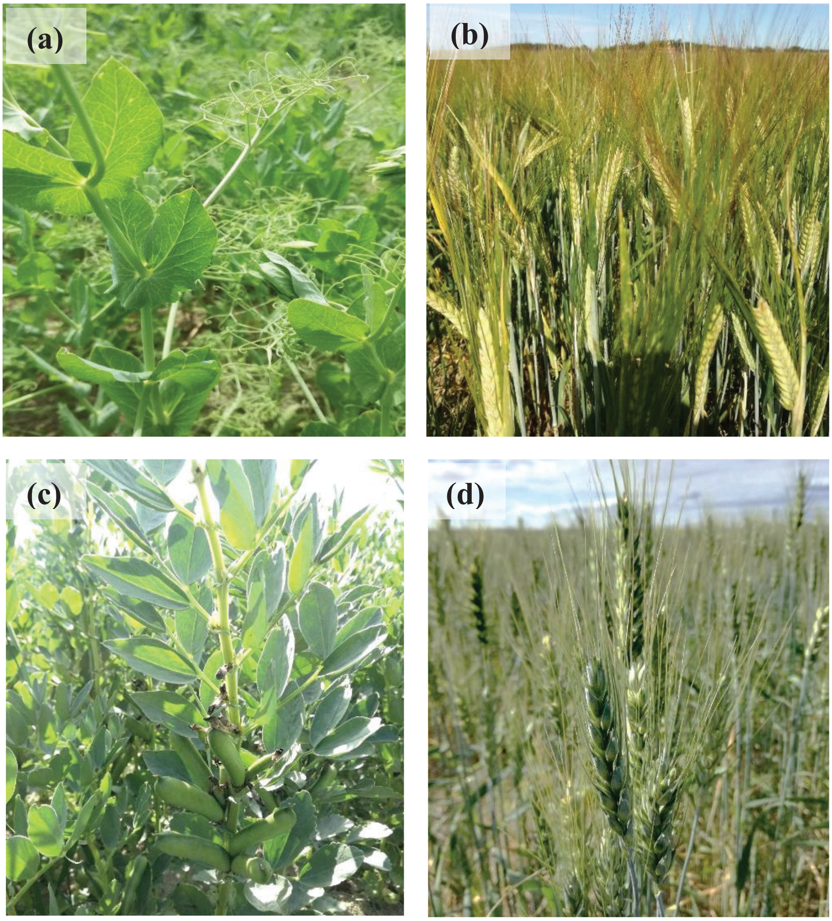


Figure 3. Pictures of the four crops; pea (a), barley (b), faba bean and wheat (d) used in this thesis. The crop species were grown as sole crops (as in the picture) and as two-species intercrops of pea-barley and faba bean-wheat. Two cultivars of each legume and three cultivars of each cereal were used. Photos: James Ajal.

3.1.2 Field study

Two intercropping experiments were established in the field in Uppsala, Sweden–SE (59° 50' 6" N 15° 42' 0" E) and Taastrup, Denmark–DK (56° 40' 7" N 12° 18' 20" E) during the spring of 2017 and 2018. The plots (measuring 10 × 2 m) were established under different input management and diversity levels. The management was categorized as "Low" and "High", with "Low" corresponding to 0 kg N ha^{-1} (SE) and 20 kg N ha^{-1} (DK). "High" corresponded to 90 kg N ha^{-1} for pea-barley and 140 kg N ha^{-1} for faba bean-wheat (SE). In DK, the "High" corresponded to 60 kg N ha^{-1} and pesticides. The different crop species (or cultivars) were sown as sole crops and intercrops (i.e., two diversity levels) in a replacement design with the intercrops having 50% of the proportion of plant population in the sole crops. The plots were arranged in a split-plot design with management as main plots and the different species combinations randomized within the subplots (Fig. 4). To evaluate N availability to the crop and weed, micro-plots (dimensions 0.8 × 0.5 m) with ^{15}N labelled ammonium nitrate ($^{15}NH_4{}^{15}NO_3$) were established in pea-barley plots with 0 kg N ha^{-1} (for details, see Fig 1 in paper III). The overview of the plots is illustrated in Fig. 5.

3.1.3 Pot study

Complementary to the field experiments in SE, a pot experiment was established in Ultuna, Uppsala, SE (59°49'01.3"N 17°39'26.0"E) in an enclosed net yard in the spring/summer of 2019 (Fig. 6). Since the experimental site was not fully enclosed, plants experienced similar weather conditions (for example, air temperature, light, and rainfall) as in the field. Two cultivars each, for faba bean and wheat, were selected from the list of cultivars previously used in the field experiment in 2017 and 2018. Weed plants (*C. album)* used in the experiment were transplanted from a naturally growing weed population in the vicinity of the experimental site. Similar to the field experiment, the pots were arranged in a split-plot design with the weed treatment (i.e., pots with crops grown together with the weed) as the main plot and the different cultivar/species combinations as subplots (Fig. 7). The plants were watered when required and a nutrient solution of 2 ml l^{-1} containing mostly N-P-K (proportions 51:10:43), but also S, Ca, Mg, and microelements in small proportions, were added to each pot at least once a week.

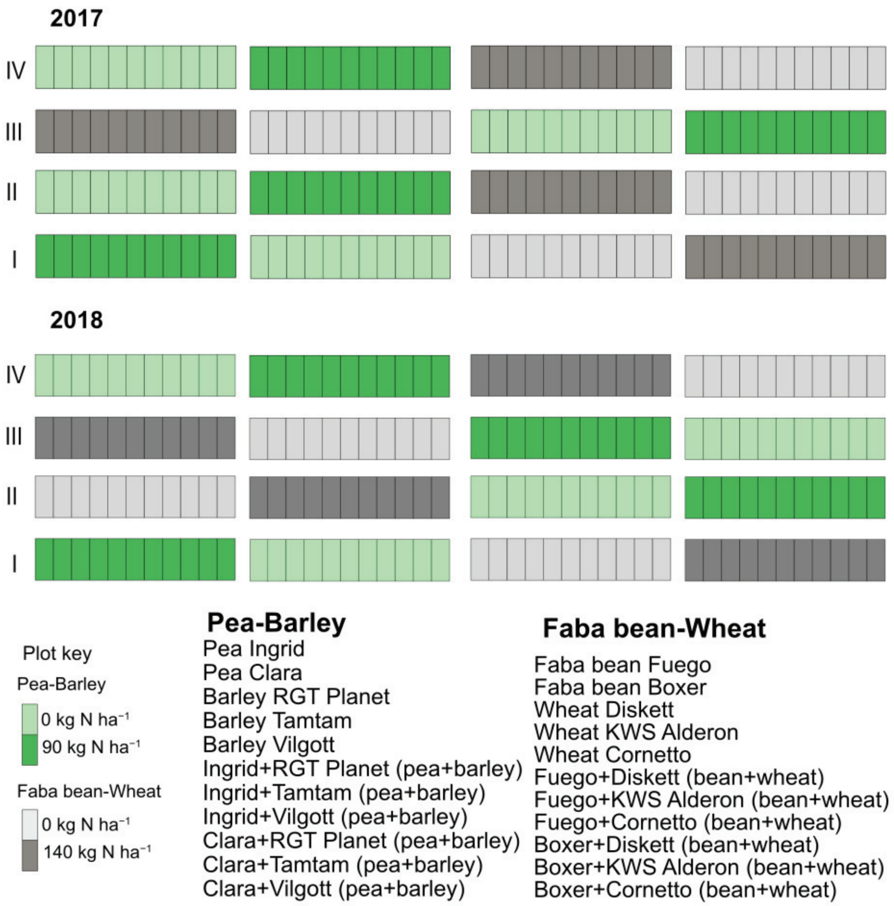


Figure 4. The layout of field experiments that were conducted in Uppsala, Sweden in 2017 and 2018. The plots colored green are pea-barley, while faba bean-wheat are in plots colored gray. The different color shades represent different nitrogen levels. Each plot measured 10 × 2 m. The field design was modified from Fig. 1 in paper III.

Figure 5. A section of the field experiment showing the layout of the plots (a). The plots show sole crop barley (left foreground), sole crop pea (centre foreground) and pea-barley intercrop (right foreground). (b) shows a section of the field at flowering stage with sampling ongoing and (c) shows a section the field at crop maturity in preparation for grain harvesting using a combine harvester. Photo (a) was taken in 2017 and photos (b) and (c) in 2018. Photos: James Ajal.

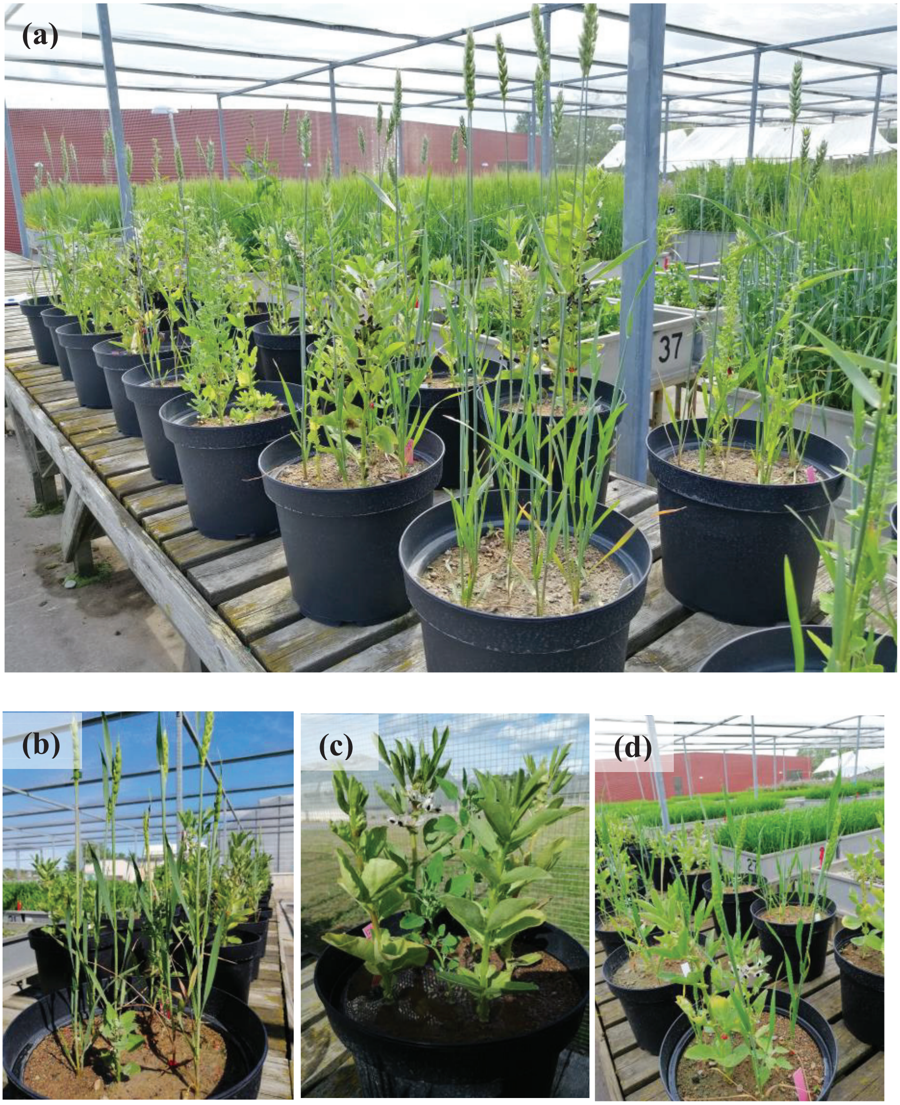


Figure 6. Overview of the pot study conducted under semi-controlled conditions (a). (b) shows wheat in sole crop grown with weed (*Chenopodium album*), (c) faba bean sole crop grown with weed, and (d) a mixture of wheat and faba bean grown with weed. Photos: James Ajal.

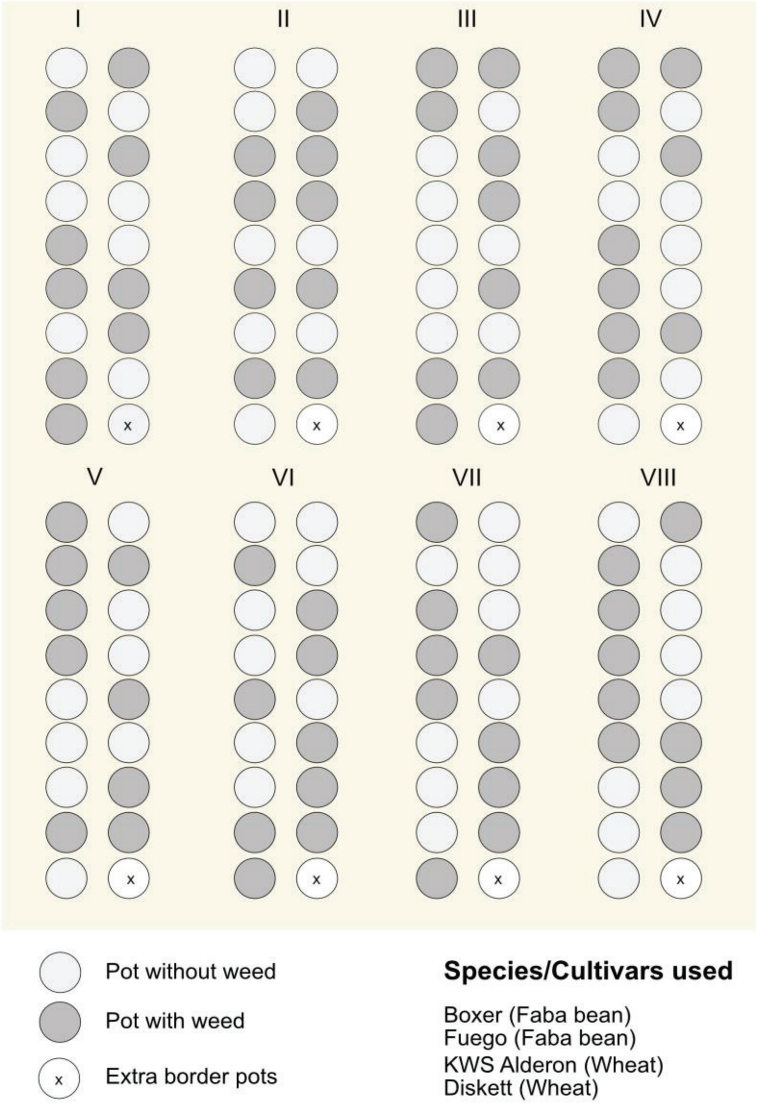


Figure 7. The layout of the semi-controlled pot study that was conducted in Ultuna, Sweden, in 2019. Each circle represents a plastic pot of diameter 26 cm and a volume of 7.5 l in which plants were grown. Each of the cereal and legume cultivars was grown in a pot as sole crops and as two-species mixtures. Numbers I-VIII shows the blocks in which weed treatment was randomized. Illustration: James Ajal.

3.2 Measurements and biomass sampling

During the experimental periods, various measurements were performed in both the field and pot study. To ascertain the soil physical properties and nutrient content of the field sites, soil samples were taken and analyzed at the start of each experimentation period. During the plant growth period measurements were performed at different plant growth stages, and destructive biomass samplings were done at crop flowering and maturity for both the field and pot studies. Measurements were assessed at the plot level, on an area basis, and at individual plant level (Table 1).

Table 1. Summary of different measurements performed in the field and pot experiments. Measurements were categorized into 'non-destructive and 'destructive', and the level they were measured.

Non-destructive harvest	**Destructive harvest**
Individual plant basis	*Individual plant basis*
Plant height (cm plant^{-1})$^{\#}$	Leaf dry weight g plant $^{-1}$)
Development stage (BBCH-scale)	Stem dry weight (g plant $^{-1}$)
Tiller /branch number (plant $^{-1}$)	Seed weight (g plant $^{-1}$)
Canopy height (cm)*	Head/pod weight (g plant^{-1})$^{\#}$
	Weed biomass (g plant $^{-1}$)
	Leaf area index, LAI (m^{2} m^{-2})*
	Specific leaf area, SLA (cm^{2} g^{-1})*
Area/pot basis	*Area/pot basis*
Ground cover (%)*	Root dry weight (g pot)$^{\#}$
Flowering date (days)	Shoot biomass (g m^{-2})*
Leaf area index, LAI (m^{2} m^{-2})*	Weed shoot biomass (g m^{-2})*
	Grain yield (t ha^{-1})*

Measurements performed only in the field study are marked with an asterisk (*) and those only done in the pot study are marked with a hash (#)

For the destructive biomass harvests in the field, crop plants and weeds were cut at the soil line from an area of 0.5 m^{2}, subdivided in two 0.25 m^{2} at each end of a plot, and in addition, 5 individual plants of each species were taken. This sampling procedure was done at crop flowering and repeated at crop maturity. At maturity, crops for grain yield measurements were harvested using a combine harvester from the central 12 m^{-2} dedicated for grain yield assessment. In the laboratory, crop plants in the intercrops were separated per species, and the weeds separated into the two dominant weed species (i.e., *C. album* and *Galeopsis spp.)* and 'other weeds' (Paper I, II and III). For the pot study, plants from each pot were cut and separated per species, and the roots from each pot were washed under running water. All biomass samples were dried at 70°C for 48 h. Dried biomass samples from five individual plants of each species were pooled together and N contents of the shoot and grains were analyzed (field experiment). In the pot experiment, N content of the shoot and grain were analyzed from individual plants of each species pooled together, and for the roots, N content analysis was performed per pot.

The grain yield at harvest was used to assess crop productivity in the sole crops and intercrop, and to calculate the Land Equivalent Ratio (LER) (Mead and Willey, 1980; Weigelt and Jolliffe, 2003). Thus, the LER evaluates crop productivity in terms of the relative amount of land area required to produce the same yield in the sole crop *vs.* intercrop. $LER = (Ya_{ic}/Ya_{sc})+(Yb_{ic}/Yb_{sc})$, where Ya and Yb is the yield of species a and b, respectively, and *ic* and *sc* correspond to intercrop and sole crop, respectively. Values of $LER>1$ imply a higher yield advantage of the intercrop than in the sole crop, and $LER<1$ shows less yield advantage in intercrop than the sole crop.

3.3 Nutrient analysis and calculations

3.3.1 Nitrogen availability to the crop and weeds

^{15}N isotopes were analyzed from the dry biomass samples of the crop and weeds. We used barley grown in a sole crop as a reference crop, which was used in the calculation for N available to the crop and weed. The amount of N available to the crops and weeds were estimated as follows; the proportion of N that was derived from air/fixation (Ndfa %) was estimated using both the natural abundance by Shearer and Kohl (1986) and isotope dilution methods (Fried and Middelboe, 1977). The amount of N derived from fertilizer (Ndff %) was calculated from N in the plant sample and fertilizer, and N from the soil (Ndfs %) was then subsequently calculated by subtracting Ndfa % and Ndff % from the total N (paper III).

3.3.2 Nitrogen accumulation efficiency and its components

Nitrogen economy in the different species was evaluated in form of N accumulation efficiency, NAE (Weih et al., 2011; Weih et al., 2018) (papers I and IV). The NAE was calculated based on the plant N content and the N pools accumulated in the different plant organs. It is a product of mean N uptake efficiency (U_N), grain specific N efficiency ($E_{N.g}$) and grain N concentration ($G._{N.g}$), as illustrated in Fig. 8.

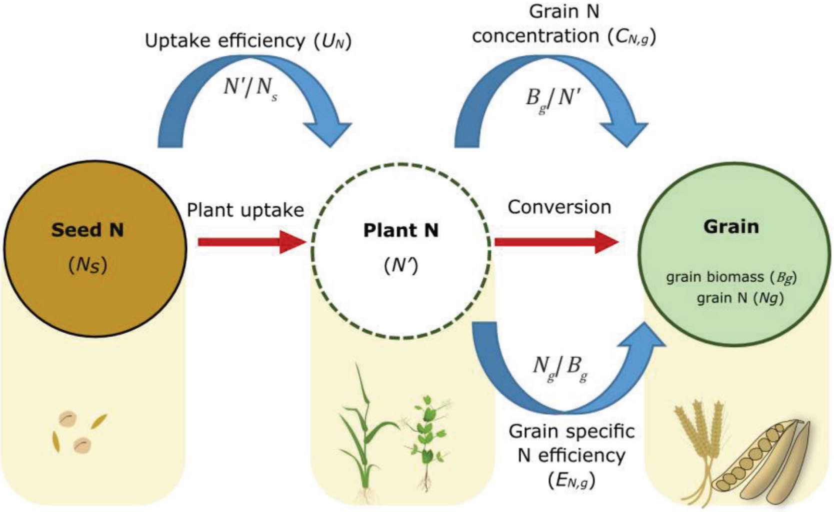


Figure 8. The relationships between different N accumulation efficiency (NAE) components showing how N in the seed grain is transformed during the plant growth period to N in the harvested grain. The red arrows represent the major factors contributing to the overall NAE, and the blue arrows represent the drivers for N uptake and conversion with a plant. Acronyms: U_N -Mean N uptake efficiency, $E_{N.g}$ - Grain specific N efficiency, $C_{N.g}$ - Grain N concentration, N' - Mean plant N content during the entire growing season, N_s - initial N content in the grain seeds, B_g -Grain biomass at harvest, and N_g - N content of the grain. The conceptual idea is based on Weih et al. (2011; 2018). Illustration: James Ajal.

3.3.3 Analysis of other macronutrients

For the plants grown in the pot experiment (paper IV), wheat and faba bean phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) contents were analyzed from shoot biomass sampled from the sole crop and intercrop pots at flowering. The nutrient concentrations were used in the calculation of scaling exponents to establish the relationship between the relative concentration of N and P on the one hand, and the other nutrients (K, Ca, Mg, and S) on the other hand, based on the niche volume concept by Ågren and Weih (2020). Thus, stoichiometric niche volumes were calculated separately for N and P (V_{NP}), and the other nutrients (K, Ca, Mg, and S)(V_{Oth}), which were used to determine the scaling relations $V_{Oth} = \beta(V_{NP})^{\alpha}$; in which α represents the scaling exponents and β the intercept. A value for $\alpha > 1$ corresponds to a higher relative increase in the concentration of other nutrients compared to N and P, while $\alpha < 1$ denotes a lower relative increase in the concentration of other nutrients compared to N and P (Ågren and Weih, 2020).

3.4 Trait space analysis

Functional trait spaces were analyzed for selected cultivars of each species in the pea-barley and faba bean-wheat species combinations. Hypervolumes were constructed in the ‘hypervolume’ R package by Blonder and Harris (2018) using the Gaussian kernel density estimation method detailed in Blonder et al. (2014) and Blonder et al. (2018). Based on the analysis goal, different traits were selected and constituted the axes of multi-dimensional hypervolumes (papers I and II).

3.5 Statistical analysis

Statistical analyses were performed using R, versions 3.5.0-4.0.2 (R Core Team, 2020). Depending on the parameter, different models were used. Data were checked for compliance with assumptions for normal distribution. This was done using either Levene’s tests or through Quantile-Quantile plots. Data that violated the assumptions were log-transformed before analysis of variance (ANOVA) was done, or non-parametric methods were used. For evaluating the effects of various factors on plant biomass, grain yield, and the NAE components, linear mixed effect models [nlme Package: ‘*lme*’ by

Pinheiro et al. (2017)] were used. Where cultivar identity was considered as a factor in the analysis, management (N-level), diversity, cultivar, and their interactions were used as fixed effects, and management nested within blocks (replicates) was treated as random effect (paper I). The same model as above was used in the pot experiment, but in place of management, the weed treatment was used in the model (paper IV). In the crop-weed analysis (paper III), sampling time, crop, weed species, and management were used as fixed effects, and block or main plot were designated as random effects, depending on the aim of the analysis. One-way and two-way ANOVA were used to analyze trait spaces of the different species in the sole crop and intercrops (papers I and II). All the ANOVA was followed by Tukey's multiple comparison tests to determine the differences between the treatments. Significant differences were considered at $\alpha=0.05$.

4. Results and Discussion

In this thesis, different approaches were used to analyze both the data and plant samples for the cereals and legumes, grown as sole crops and intercrops. Based on the multi-dimensional trait space approach, I found that the cultivar choice (i.e., the identity of the cultivar) had a stronger effect in shaping the functional trait space than the diversity level. In another instance, based on a different set of functional traits and environmental conditions, the diversity level was an important influencer of trait space (papers I and II). In addition, I found that the relative competitiveness and dominance of two weed species, common in Swedish fields, was influenced by the gradient of N availability initiated by pea and barley in sole crops and intercrops (paper III). In paper IV, I showed under semi-controlled conditions, that a species grown in intercrop accumulated more N than the same species grown as a sole crop; and the relative accumulations of nutrients other than N and P depended on the plant neighbor.

These results were based on both field and pot experiments. The combined field and pot experiments allowed a more general evaluation of the different species under more realistic conditions, and to test specific hypotheses under more controlled conditions, for example, for root assessment. Belowground plant traits remain understudied to date, despite their significance in resource acquisition and maintaining ecosystem services, among other functions (Freschet et al., 2021; Shekhar et al., 2019). New approaches for root phenotyping using high throughput methods have been developed (Price, 2016), but separating roots in intercrops or species mixtures remains a challenge. In this thesis, root biomass was estimated from the shoot biomass of the same species, based on the assumption that the proportion of shoot and root is not changed when species are grown as sole crop and in an intercrop. I recognize the limitation with this approach, but consider it minimal to significantly influence the results of the study.

4.1 The role of intercropping in shaping plant interaction and functional trait spaces

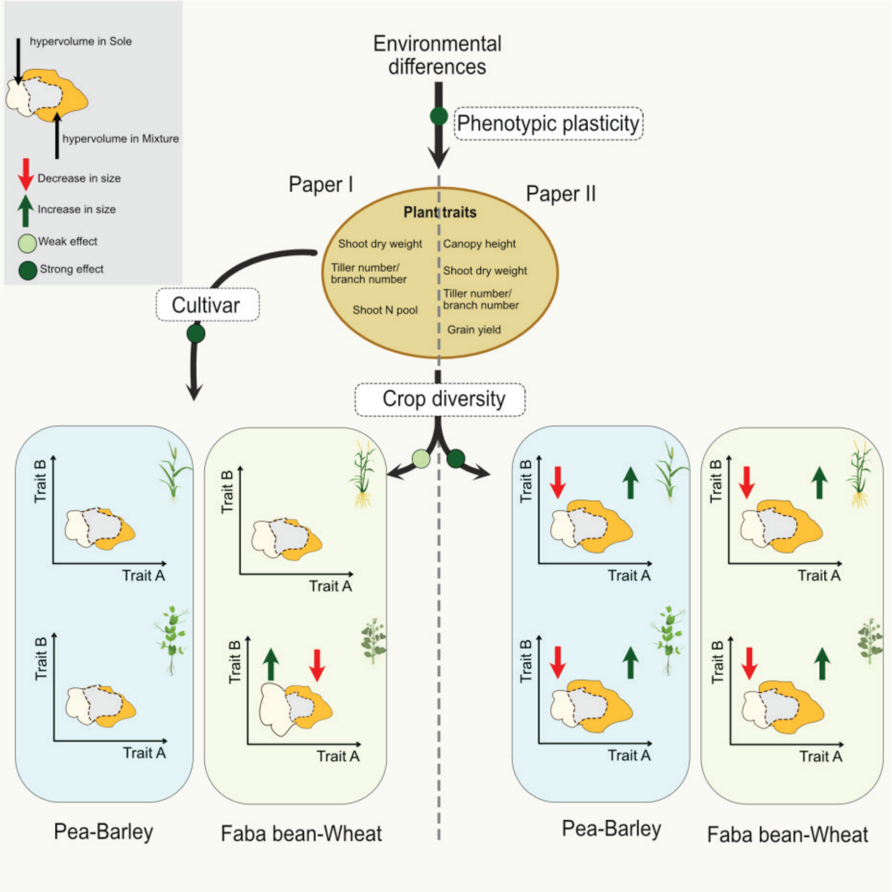


Figure 9. Conceptual diagram summarizing the main findings related to functional trait space and phenotypic plasticity in sole crops, and two-species mixtures of pea-barley and faba bean-wheat. These findings are based on papers I and II of this thesis. Growth conditions in the different environments and diversity in the mixtures influenced trait spaces of the different crop species. The different cultivars resulted in a stronger effect of cultivars in one case, and a stronger effect of crop diversity (diversity level) in another. Illustration: James Ajal.

Studies that focus on plant species interaction, especially in intercrops, often rely on mean trait values of biomass or grain yield to assess productivity. Here, I used a combination of mean trait values and variations of multiple traits to measure functional trait spaces. The latter add an important dimension, because species interaction is influenced by the functional traits of the involved species, which in turn defines how these species make use of the available resources in the environment in which they are grown (Benavides et al., 2019; McGill et al., 2006). In this thesis I have shown that, based on results in paper II, environmental differences drive phenotypic plasticity, hence different expressions of traits based on the environmental conditions in the trial locations (i.e., SE and DK). The environment, in this case, was taken in a broad context, referring to the combined effect of weather, soil conditions, and management that characterize growth conditions in the different locations.

As expected, the diversity level influenced functional trait space in my experiment, indicated by differences in hypervolume sizes and other measures of hypervolume overlap, which were found for species in the different diversity levels. Particularly, I found that the different crop species had larger hypervolumes in the intercrops than in the corresponding sole crops (Fig. 9; Fig. 1 and Table 2 in paper II). However, in some cases, the effect of diversity level was less apparent and instead, the cultivar identity was more important (example in paper I). The changes in hypervolume sizes are associated with the difference in intraspecific trait variability at the different diversity levels – more variable in the mixture than in sole crops, as I have indicated in most cases. I have previously highlighted the different expressions of plant traits with respect to results from SE and DK, but the difference in trait variation within each species may also be explained by phenotypic plasticity in response to heterospecific neighboring plants (Helsen et al., 2020). Growing species as intercrops in contrast to sole crops can also alter resource availability, for example, the amount of N that may be available for uptake by the neighboring plants (paper III). In general, in this thesis, and other previous studies, it is challenging to disentangle the role played by phenotypic plasticity, for example in root traits when plants are growing under different soil N or water status, and the actual effect of facilitation or complementarity in the mixture. In the cases where I found a stronger effect of the cultivar identity than the diversity level, the dissimilarity of functional traits of the different cultivars could have resulted in more intraspecific trait variation caused by phenotypic plasticity (paper I).

Both observations of diversity level and cultivar identity having strong effects in the different scenarios (papers I and II) are valid and conform to the theory that different functional traits facilitate the occupation of different niches. This niche theory is normally applied in the context of species coexistence in which species specialized functional traits are ‘designed’ for specific niches (Sterck et al., 2011). There were some cases where the same cultivars were used but different observations in trait space were observed in papers I and II. One explanation for this discrepancy could be the difference in the set of functional traits considered in the analyses of trait spaces. In this thesis, cultivar identity had a stronger influence on trait spaces when traits related to N uptake and utilization were used (paper I), and diversity level had a stronger effect in shaping trait spaces when traits related to aboveground resource acquisition were used (paper II). A previous study by Bittebiere et al. (2018) showed that different traits can have different relative contributions than other traits in the shaping of multidimensional trait spaces, but also when single or multi-species assemblages are used.

4.2 Species-specific N economy in sole crops and intercrops of cereals and legumes. Are biomass and grain yield affected?

Determining differences, for example, in N accumulation or grain yield of crop species grown at different diversity levels is useful in evaluating crop performance in the sole crops *vs*. intercrops. Indeed, many studies have used this approach, coupled with various other indices that measure competition in plants (Corre-Hellou et al., 2011; Wang et al., 2014; Weigelt and Jolliffe, 2003). In this thesis, I measured N economy not only as differences in the N accumulation efficiency components at the different diversity levels, but also the size of the differences by calculating effect sizes (ES).

Based on the ES values, intercropping had large or moderate effects on N uptake efficiency (U_N) in all crops, although the direction of the effects contrasted with species (Fig. 10). The cereals particularly had higher U_N in the intercrops than sole crops, while the legumes’ U_N were lower in the intercrops (Fig. 10 a). At the cultivar level, intercropping also had moderate to large effects on U_N for most of the cultivars except for a few that had no or negligible effects on U_N. Based on absolute U_N values, I found that N uptake values of selected cultivars of wheat in the sole crops, or when intercropped with faba bean, were consistent in the field and pot experiments – wheat had higher U_N in the intercrop than in the sole crop (papers I and IV). Overall, the range of U_N values in the pot study (paper IV) was similar to that of the field study (paper I) in 2018, a year with relatively warmer and drier weather than the mean of the historical records of the region. In 2017, the U_N values for the field study were relatively higher than the pot study. The similarity in my pot and field studies (the year 2018) could be associated with the soil water content. The pot study was conducted in sandy soils that readily drained irrespective of the outdoor temperature, and on some occasions, the soil in the pots was dry despite the regular watering. This was similar to the conditions experienced in the field in 2018, where plants experienced long periods without water.

The trend in ES values for U_N shown at both crop and cultivar levels was similar to that of NAE (Fig. 10 d). This is also consistent with observations in paper I of this thesis (Fig. 6), where and I found a positive correlation between U_N and NAE for both the cereals and legumes. Only small effects (low ES values) of intercropping were found for E_N and C_N (Fig. 10 b and c).

The large ES values for U_N and NAE confirmed that both the cereals and legumes are influenced by intercropping, but their performance, for example, in making use of limited resources or biomass accumulation may be differently affected. I have shown in papers III and IV that the more competitive nature of the cereals gives them an advantage over the legumes for both soil and aboveground resources when grown in the intercrop. While the better performance of cereals in the intercrop that I have demonstrated in this thesis is not new, as other studies previously reported similar observations (Corre-Hellou et al., 2006), the finding that plant N uptake efficiency (U_N) is a major driver for the observed better performance of the cereals *vs.* legumes than conversion efficiency ($E_{N,g}$) is interesting. Based on the trait space analysis shown in paper I of this thesis, larger trait spaces (larger hypervolume sizes with less overlap) of the cereals were associated with increased U_N, which implies that the greater variation in functional traits of the cereals facilitated the uptake of more N in the intercrop. A review by Stomph et al. (2020) also pointed out acquisition efficiency (reflected by U_N in this thesis) as being of greater significance in intercrops than conversion efficiency (reflected by $E_{N,g}$). In another study involving dicots and monocots, diversity in the mixtures increased seed yield but reduced the harvest index – the ratio of seed yield to total aboveground biomass (Chen et al., 2021).

To further evaluate the effect of N economy of the different species grown at the different diversity levels, I compared the productivity of the crops. I found a few instances where the land equivalent ratio, LER (based on grain yield) was greater than 1, i.e., a yield advantage in the intercrop compared to the sole crop (Fig. 11). The lower partial LER of the legumes compared to the cereals was the major contributor to the LER<1 in most cases. For the study system used in my thesis (species and cultivars), the cereal yield did not compensate for the underperformance of the legumes, an observation that has been reported in previous intercropping studies including cereals and legumes (Elhakeem et al., 2019; Raseduzzaman and Jensen, 2017). Instead, I found that the more competitive cereals may have suppressed the less competitive legumes in the intercrop, leading to lower partial LER in the legumes than the cereal. Pelzer et al. (2012) previously found a similar observation. In contrast to my expectation, the amount of N (N level) applied to both the pea-barley and faba bean-wheat mixtures had no effect on LER (Fig. 11).

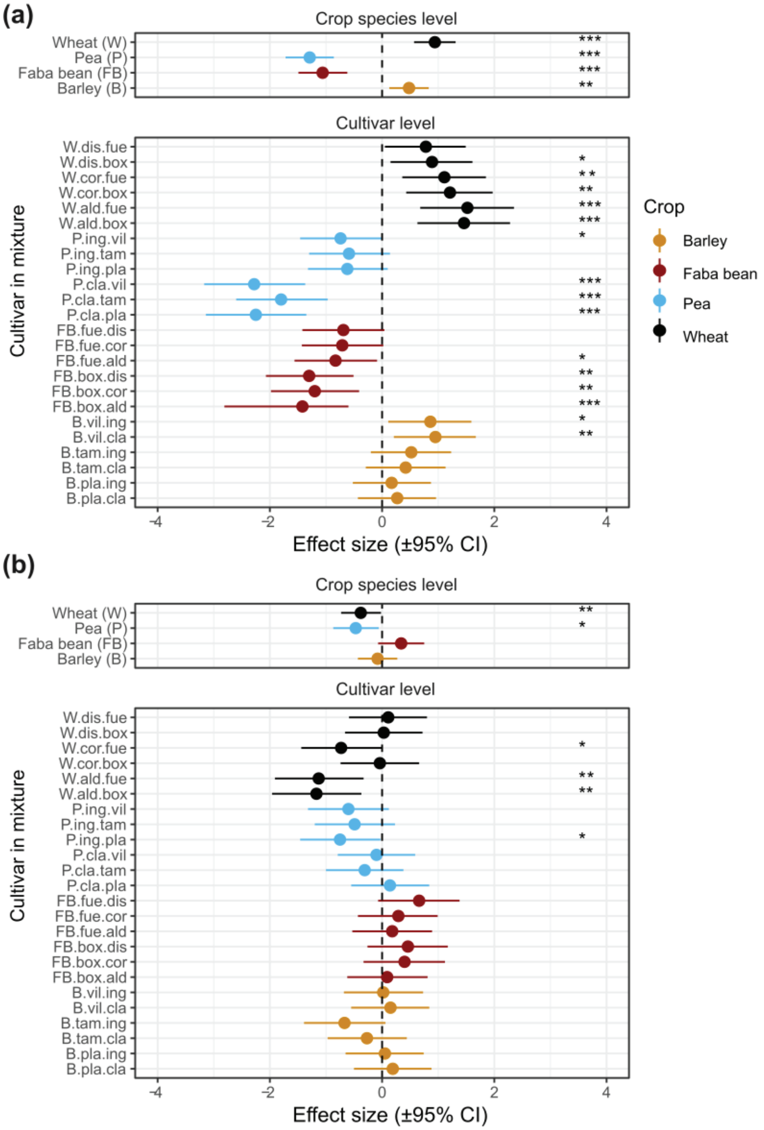
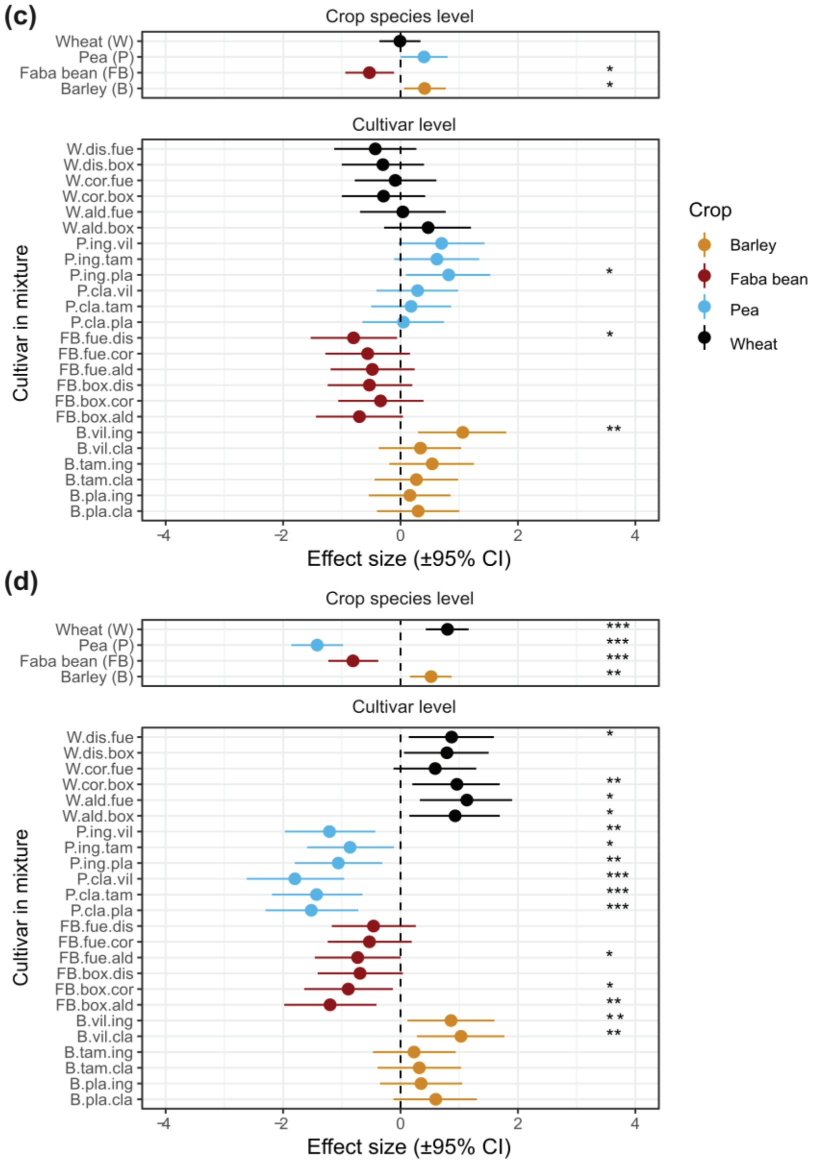


Figure 10. Effect sizes for (a) Mean N uptake efficiency, U_N, (b) Grain specific N efficiency, $E_{N.g}$, (c) Grain N concentration, $C_{N.g}$ and (d) N accumulation efficiency, *NAE*. Effect size was calculated at two levels; first for each crop species in the sole crop and intercrop, and second for different cultivar combinations of each species. Figures were based on pooled data for 2017 and 2018 in which papers I, II, and III are the basis. The error bars are 95% confidence intervals at α=0.05. Symbols showing significance levels;*** = P≤ 0.001; ** = P≤ 0.01; * = P≤ 0.05 following a linear mixed effect model analysis.



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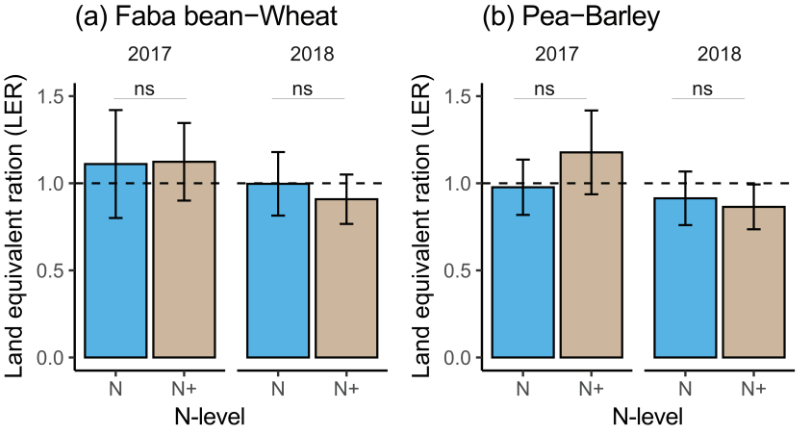

Figure 11. Land equivalent ratio (LER) values based on partial LER for faba bean and wheat (a) and partial LER for pea and barley (b). The LER was calculated from the grain yield at harvest in the 2017 and 2018 growing seasons. The data presented were used in papers I, II, and III. The LER values above one (LER>1) imply yield advantages of the intercrop compared to the sole crops based on the land area. The species combinations were grown under two nitrogen (N) treatments. *N* corresponds to 0 kg N ha^{-1} for both species combinations while *N*+ corresponds to 140 kg N ha^{-1} and 90 kg N ha^{-1} in the faba bean-wheat and pea-barley species combination, respectively. The error bars are 95% confidence intervals at α=0.05. ns= non-significant.

4.3 Crop-weed interaction, weed suppression and N accumulation in crops and weeds

Agricultural practices today are heavily dependent on synthetic herbicides as a means to control weeds, which can have detrimental effects on the environment (MacLaren et al., 2020). There is currently a drive for a paradigm shift towards more ecological methods to manage weed populations (Davis and Frisvold, 2017; Walsh et al., 2013). In this thesis, I used intercropping as an integrated approach for managing weeds, and showed that intercropped cereals and legumes offer a stronger reduction in the amount of N available to the weeds than the same crops grown as sole crops, especially for the legumes. The reduction in the amount of N available to the weeds is crucial, because weeds typically depend on the soil N at their disposal, to accumulate more biomass. This relationship between the available N and weed biomass was illustrated both in the field and the pot study, where I showed a positive correlation of accumulated weed biomass and the amount of N available for uptake by the weeds (Fig. 4 in paper III, field), and a negative relationship between the accumulated crop N pool and weed shoot biomass (Fig. 4 in paper IV, pot). Although I used different species combinations; pea-barley and faba bean-wheat in paper III and IV respectively, the available N-weed biomass relationship was consistent. I demonstrated in papers III and IV that intercrops are more efficient in acquiring soil N than the respective sole crops resulting from the complementary N uptake from the larger explored soil volumes in the intercrop, or from different N sources. These results are consistent with the findings by others (Tang et al., 2021). At a global scale, Jensen et al. (2020) have shown that intercropped cereals and legumes are capable of utilizing more soil N resources and hence reduce losses to the environment through leaching, emissions, and other processes.

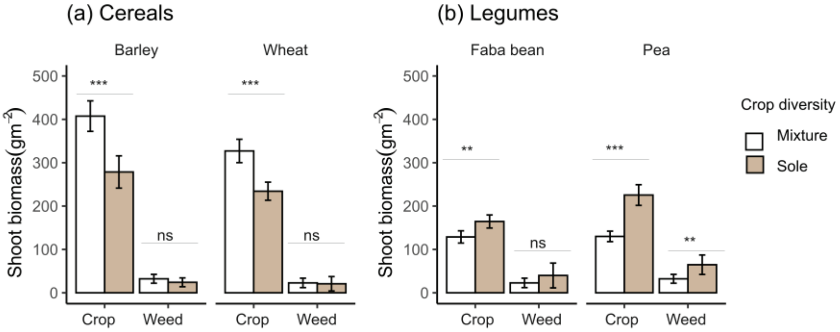

Figure 12. Crop and weed biomass accumulation of different crops grouped as cereals (a) and legumes (b). The figures are based on data for 2017 and 2018, which form the basis for papers I, II and III. The crops were grown as sole crops and as two-species intercrops of pea-barley and faba bean-wheat. The shoot biomass of the crop was based on data pooled for all cultivars and years. The weed biomass was based on the combined biomass for all species. The error bars are 95% confidence intervals at $\alpha=0.05$. Symbols showing significance levels;*** = $P\leq 0.001$; ** = $P\leq 0.01$; * = $P\leq 0.05$; ns= non-significant.

Unlike other studies, where the presence of cereals in the intercrop markedly reduced the amount of weed biomass, for example in Bedoussac et al. (2015) and Corre-Hellou et al. (2011), I found no difference in the amount of weed biomass between the cereals that were grown as sole crops or intercrops (Fig. 12). However, the weed biomass was lower in the intercropped than the sole cropped pea. The crop biomass of different species indicated by results in this thesis may be explained by factors other than competition for aboveground resources. Generally, sole cropped peas are known to have a lower competitive advantage against weeds than when intercropped with cereals (Bedoussac et al., 2015; Hauggaard-Nielsen et al., 2001).

When I considered the accumulation of other nutrients in the pot study (paper IV), I found that the identity of plant neighbor (crop species or weeds) changed the scaling exponents (V_{Oth} *vs.* V_{NP}) in some cases, implying different relative accumulation of nutrients other than N and P (Ca, K, Mg, and S) compared to the accumulation of N and P. For the legumes, the difference in scaling exponents may be associated with the need to accumulate more of the other nutrients (Ca, K, Mg, and S), since they can rely on biologically fixed N. When the legumes were grown together with weeds, the high competition for N and P resulted in less accumulation of N and P and greater accumulation of Ca, K, Mg, and S - possibly as a nutrient compensation mechanism.

In summary, the results from this thesis provide evidence that intercropping stands out as a promising option to improve the competitiveness of crops such as pea against weeds, thereby offering an alternative to using chemical weed control.

5. Conclusions and recommendations

I applied ecological principles in this thesis to investigate the potential of intercropping for increasing resource use efficiency, improving crop competitiveness against weeds, and overall crop productivity. This is timely considering the need for the adoption of more sustainable crop production methods to meet the current scenarios for future global climate and environmental changes.

In this thesis, I have shown that intercropping, cultivar identity, and environmental differences influence functional trait space in cereals and legumes. The differential expression of functional traits (i.e., different trait values in the sole crops *vs.* intercrops) is achieved through phenotypic plasticity. It is also worth noting that the set of functional traits used in the analysis may have different influences on trait space. Therefore, when designing intercrops, it is recommended that cultivars should be chosen based on their functional traits, especially those that complement each other when grown in mixtures.

Improved N uptake efficiency that facilitates greater N acquisition explains the competitiveness of the cereals for soil resources. The acquisition of N was more important than N conversion to grain yield. On the positive side, this can facilitate more N capture by the mixture, but in some cases, the cereals can negatively affect the legumes' performance by dominating the legumes when grown in an intercrop.

Soil N input in the form of fertilizer increases the amount of N available for uptake by the crop, but also to the weeds that compete with the crops for the same resources. In fact, *C. album* (characterized as highly responsive to N) benefitted more from the fertilizer N addition than the crop. It is recommended that, as a routine, the soil N status should be determined prior to any fertilizer addition and, when soil N is above suboptimal level, fertilizer addition should be forfeited to avoid a scenario where weed growth is favored over crop growth.

6. Future prospects

Ecological principles underlying intercropping may be cross-cutting for different species combinations or environments, but its application is context-dependent and should be considered on a case-by-case basis. For example, complementarity and facilitation are documented mechanisms for higher productivity in intercrops than sole crops, but their dynamics may vary in different conditions; take for instance nutrient-rich soil *vs.* nutrient-poor soil. Evidence from literature and some results from this thesis show that under certain conditions, intercrops are more competitive and therefore better at suppressing weeds than the sole crops, especially the legumes. The results of this thesis also showed, based on two weed species that the dominance and competitiveness may change with N fertilization level. Future studies in areas with a more species-rich or diverse weed flora than we used in our study are necessary to evaluate patterns in weed competition and dominance. Based on the results of this thesis, it is still unclear how species abundance (i.e., the number of individuals within each weed species) would affect the individual weed species competitiveness or dominance at the different diversity levels. This remains to be investigated.

For both the field and pot studies, N acquisition was higher in the intercrops while N conversion was similar for most cases in the cereals, which could imply less N investment in the grains. Chen et al. (2021) also highlighted this issue in the reduced harvest index of the mixtures. From this background, increasing conversion efficiency appears to be a promising means to further improve the productivity of intercrops. Field or greenhouse studies that focus on senescence (or stay green) traits in intercrops, particularly while providing environmental conditions that prolong senescence in intercrops, are necessary. In the long term, there is a need to determine the genetic variation in traits that are key in plant N re-allocation or conversion, which is necessary to inform future breeding programs for intercrops.

The results from the pot study of this thesis have shown that plant nutrient stoichiometry is affected by the plant neighbor, which may be relevant for understanding the (co)-limitation of other nutrients. Research on resource use efficiency, hitherto focused on N and P, needs to be expanded through further studies on the effects of intercropping on resource accumulation of other nutrients.

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Popular science summary

In the past decade alone, we have witnessed the mass media flooded with several climate disasters that range from floods, prolonged droughts, heatwaves, with almost every subsequent year taking the trophy for the highest recorded temperature. The signs are clear! We are living in a period of changing climate which has threatened the ecosystem directly, and food security indirectly. The threat to food security is exacerbated by the growing global human population that is currently estimated to be about 7.8 billion, meaning a large population may fail to attain a level of food self-sufficiency.

For any problem, a solution starts with knowing the root causes. Therefore it is worth acknowledging that the food system itself is a major contributor to global emissions. Whereas reversing this worrying effect of climate on food production is challenging, there is a lot that can be done to mitigate these effects. One step towards mitigating these effects is by re-thinking how we do agriculture. Today, large areas of agricultural land in the developed countries are occupied by sole crops – where one crop type is grown at a time. The management of such croplands is also characterized by the use of heavy machinery to do farm operations, addition of inorganic fertilizers to supply crops with nutrients, and other chemical inputs like fungicides and herbicides to control diseases and weeds, respectively. The aforementioned management practices may be effective for their intended use, but in the long term, they are not sustainable and negatively affect the environment. Alternative crop production methods, based on ecological principles, which deliver the most benefit from the supplied resources (increased resource use efficiency) will significantly reduce the reliance on chemical inputs. One such method is intercropping, where two or more crops are simultaneously grown on the same land.

In my thesis, I used two crop types, cereals (wheat and barley) that belong to the grass family, and legumes (pea and faba bean) that are capable of utilizing nitrogen from the air. The cereals and legumes were grown alone as sole crops and in another case, two species were mixed within the same row as an intercrop; i.e., pea mixed with barley and wheat mixed with faba bean. The advantage of growing cereals and legumes together is that the legumes fix nitrogen from the air to the soil that can be utilized by the cereal partner to grow better and produce more grains. Since cereals and legumes belong to different plant families, they also possess different plant features that allow them to acquire the same resources from different regions of shared space – the niche. For example, cereals that have a more developed root system draw resources from deeper layers of the soil while the legumes, with shallower roots, draw from the upper layers of the soil profile. Based on this background, my thesis aimed to test whether intercrops would facilitate the allocation of a larger region of the shared spaces to each species than when grown alone. In addition, I tested whether intercrops allow more efficient use of required resources for growth, especially nitrogen, to produce more grain; and whether the growth of weeds is reduced in the intercrop compared to the sole crop. I found that the kind of each species (cultivar) used in the intercrop determines how much of the shared space is allocated to each species, and intercropping together with growth conditions may change the expression of specific plant features. I have also shown that growing the cereal and legumes together works to the advantage of the cereals as they take up more nitrogen from the soil than when grown alone. In addition, the results presented in my thesis show that the weed characteristics determine how much they compete with each other and the crop for nitrogen, and the more nitrogen is added to the soil, the more the weed can take it up. In general, growing the cereal and legume together reduced the amount of weeds compared to when the legume was grown alone.

Based on these findings, my recommendation for choosing species for an intercrop lies in the specific features of each species. Specifically, one should aim to include only those with features that allow drawing resources from different regions of the shared space. Concerning the relationships between the crop and weed, I have shown that intercropping can reduce weed pressure by suppression without the need to use chemicals, especially for the legumes that are less competitive against weeds when grown alone. With the results of this thesis, I have shown the potential of intercropping in achieving the most benefit out of the applied resources, and thereby reducing the need to depend on inorganic fertilizer and other chemical inputs. The contribution of my thesis is a step in the right direction towards reducing the use of synthetic chemicals, because, however small the amount is, any chemical inputs or emissions not discharged into the environment will go a long way in reducing the impacts of agriculture on the environment.

Populärvetenskaplig sammanfattning

Bara under det sensate deceniet har vi blivit överösta av allvarliga klimatkatastrofer, så som översvämningar, långa torrperioder och värmevågor som nästan varje år slår nya temperaturrekord. Signalerna är tydliga! Vi lever i en tid av ett klimat i förändring som direkt hotar våra ekosystem, och indirekt vår matförsörjning. Hotet mot matförsörjningen förvärras av en växande mänsklig befolkning som just nu är uppskattad till 7,8 miljarder, vilket betyder att en stor andel av befolkningen kan få svårt att få tillräckligt med mat.

För att kunna lösa ett problem behöver vi veta dess orsak. Därför är det viktigt att lyfta fram att våra livsmedelssystem bidrar stort till de globala utsläppen. Mycket kan dock göras för att motverka de negativa effekterna som klimatet har på vår livsmedelsproduktion. Ett steg mot att motverka dessa negativa effekter är att tänka om när det gäller produktionsjordbruket. I dagsläget upptas stora jordbruksarealer i industriländerna av grödor odlade i renbestånd – alltså en gröda åt gången. Brukandet av dessa odlingsmarker är också karaktäriserade av användandet av tunga maskiner för olika odlingsåtgärder, mineralgödsel för näringsämnen till grödan och kemiska bekämpningsmedel för att hantera svampangrepp och ogräs. Dessa odlingsåtgärder är ofta effektiva för sitt syfte, men är inte hållbara och påverkar miljön negativt. Alternativa odlingsmetoder, som baseras på ekologiska principer, och som ger störst nytta av de resurser som gjorts tillgängliga (ökad resurseffektivitet), skulle minska beroendet av kemiska insatsmedel betydligt. En sådan metod är samodling, där två eller fler grödor odlas samtidigt i samma fält.

I min avhandling har jag använt två olika spannmålarter (vete och korn) som tillhör växtfamiljen gräs, samt två baljväxterarter (ärt och åkerböna) som kan fixera och använda kväve från luften. Spannmålet och baljväxterna odlades ensama, i renbestånd, och som blandningar med två arter som blandades i samma rad; korn blandad med ärt och vete blandad med åkerböna. Fördelen med att odla spannmål och baljväxter tillsammans är att baljväxterna fixerar kväve från luften som via marken kan användas av spannmålspartnern för att förbättra dess tillväxt och producera fler kärnor. Eftersom spannmål och baljväxter tillhör olika växtfamiljer så har de olika utseende och egenskaper både ovan och under marken, vilket gör att de kan utnytja resurser från olika delar av samma odlingsyta – de nyttjar olika nicher. Spannmål har till exempel ett mer utvecklat rotsystem och kan därmed ta upp näringsämnen långt ned i markprofilen medan baljväxterna har ett mer ytligt rotsystem och får sina resurser därifrån. Baserat på denna bakgrund så har min avhandling handlat om att testa ifall samodling gör att en större volym av det delade odlingsutrymmet nytjas än när grödorna odlas i renbestånd. Utöver detta har jag testat om samodling möjliggör ett mer effektivt utnytjande av de resurser som behövs för tillväxt, särskilt kväve, för att producera mer kärnbiomassa, och om ogräsen hämmas i samodlingssystem jämfört med renbestånd. Jag fann att sorten (inom en specifik art) som användes i samodlingen avgör hur mycket av det delade ytrymmet som fördelas till varje art, och att samodling tillsammans med tillväxtförhållanden kan ändra uttrycket av specifika växtgenskaper. Jag har också visat att samodling av spannmål och baljväxter gynnar spannmålen eftersom de tar upp mer kväve från marken då än när de odlas i renbestånd. Dessutom har studierna i min avhandling visat att karaktärsdragen hos ogräsen avgör hur mycket de konkurrerar med varandra och med grödorna om kvävet, och ju mer kväve som finns i marken desto större andel tas upp av ogräsen. Generellt sett ledde samodling av spannmål och baljväxter till en minsking av mängden ogräs jämfört med när baljväxterna odlades i renbestånd.

Baserat på dessa upptäckter är mina rekommendationer för artval för samodling att utgå från de specifika egenskaperna för varje art. Man bör särskilt sikta på att inkludera arter med egenskaper som möjliggör att resurser tas från olika delar av det delade odlingsutrymmet. När det gäller relationen mellan gröda och ogräs så har jag visat att samodling kan minska ogrästrycket genom ökad konkurrens och utan att använda kemiska bekämpningsmedel, speciellt för baljväxter som är mindre konkurrenskraftiga i renbestånd. Med denna avhandling har jag visat potentialen att genom samodling få ut den största nyttan av de resurser som tillförts och därigenom minska behovet av gödselmedel och kemiska insatsmedel. Bidraget av min avhandling är ett steg i rätt riktning mot att minska användningen av kemiska insatsmedel, för hur liten än mängden är, kan alla kemiska insatsmedel eller utsläpp som inte kommer ut till miljön bidra till att minska deras påverkan på miljön.

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Intercropping has the potential to increase resource use efficiency and reduce weed infestation compared to the corresponding species grown as sole crops, due to intra and interspecific functional diversity. This thesis evaluated this potential in cereals and legumes using a combination of field and pot studies. The results show that intercropping can improve crop productivity through increasing resource acquisition potential, and that weed characteristics determine their competitiveness against the crop for soil available nitrogen.

James Ajal received his PhD education from the Department of Crop Production Ecology, SLU in Uppsala, MSc in Plant Sciences from Wageningen University & Research, and a BSc in Agricultural Sciences and Entrepreneurship from Uganda Christian University.

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