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# Breeding for mixed cropping: lessons learned from a genetic analysis of pea (*P. sativum* L.) - barley (*H. vulgare* L.) mixtures

Benedikt Haug

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Breeding for mixed cropping:  
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*Analyse génétique des mélanges de pois (P. sativum L.) et d'orge (H. vulgare L.) : conséquences pour la sélection pour l'aptitude aux mélanges*

**Thèse de doctorat de l'université Paris-Saclay**

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**Thèse soutenue à Paris-Saclay, le 17 décembre 2021, par**

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**Titre :** Analyse génétique des mélanges de pois (*P. sativum* L.) et d'orge (*H. vulgare* L.) : conséquences pour la sélection pour l'aptitude aux mélanges

**Mots clés :** sélection variétale, culture en mélange, pois, orge, culture en mélange basé sur légumineuses

**Résumé :** La culture en mélange de plusieurs espèces dans le même champ permet des rendements plus élevés par unité de surface et une plus grande stabilité de ces rendements. Alors que l'impact des différentes combinaisons d'espèces est bien étudié, le potentiel d'une sélection pour la culture en mélange doit être exploré. L'objectif de cette thèse est d'étudier la variabilité génétique pour la culture en mélange et de développer des stratégies de sélection de génotypes productifs et stables en mélange en utilisant comme modèle le pois (*P. sativum* L.) et l'orge (*H. vulgare* L.). Pour cela, des connaissances préalables sont nécessaires sur (a) les aptitudes générale et spécifique au mélange (GMA et SMA) des génotypes et leur importance relative ; (b) la réaction moyenne d'un génotype à la culture en mélange (effet producteur, Pr) et son influence moyenne sur le rendement de l'espèce compagne (effet associé, As) ; (c) des plans expérimentaux efficaces pour estimer les effets GMA, Pr- et As ; (d) des caractères clés corrélés à ces effets pour la sélection indirecte.

La thèse aborde ces questions en utilisant (1) une approche par simulation, (2) une expérimentation de deux ans en deux lieux avec 32 génotypes de pois de printemps et 8 génotypes d'orge de printemps (dont des mélanges de génotypes, GMs) dans un plan factoriel incomplet avec 64 mélanges et les peuplements purs, (3) une expérience d'un an en deux lieux, comparant l'efficacité de deux stratégies de sélection indirecte.

Les résultats de la simulation suggèrent qu'un plan factoriel complet ne fournit des estimations que marginalement meilleures pour les estimations individuelles et les variances de GMA et SMA par rapport à un plan factoriel incomplet de mêmes dimensions mais omettant les  $\frac{3}{4}$  des combinaisons possibles, ce qui permet de tester plus de génotypes. La variance SMA des mélanges pois-orge était proche de 0, tandis que la variance GMA du pois était modérée. Les génotypes de pois et d'orge ont montré une interaction G x E importante avec

prédominance des interactions pois x année et orge x lieu. Les génotypes de pois à feuilles ont montré une faible stabilité des effets GMA et Pr, tandis que les mélanges de génotypes de pois (GM) montraient une stabilité élevée. Les GMA du pois et de l'orge étaient positivement corrélées avec les effets Pr mais non avec les effets As, ce qui suggère la possibilité d'améliorer les rendements en sélectionnant des effets Pr élevés dans les deux espèces. Les rendements en culture pure des génotypes de pois afila étaient corrélés avec leurs rendements en mélange. J'ai identifié quatre caractères clés favorisant le rendement en mélange des pois : vigueur précoce, biomasse en début de floraison, début de la floraison et longueur des stipules. Une sélection indirecte basée sur ces caractères a été comparée à une sélection indirecte sur le rendement en pur et à la sélection directe pour le rendement du pois en mélange (GMA). La sélection indirecte sur le rendement en pur s'est avérée plus efficace que la sélection des caractères clés (106 % contre 64 % de l'efficacité de la sélection directe).

Ainsi, pour la sélection de pois et d'orges de printemps en mélange en Suisse, je recommande (1) pour les types semi-afila d'utiliser les informations disponibles sur le rendement en pur pour planifier les croisements (ou pour la création de mélanges par les agriculteurs), (2) de sélectionner les premières générations sur la base des caractères identifiés, (3) d'utiliser un seul testeur d'orge représentatif car la SMA peut être négligée, (4) de sélectionner les génotypes avec un effet Pr élevé pour améliorer la GMA, (5) d'explorer des mélanges de génotypes de pois variables pour les caractères morphologiques (présence de feuilles, longueur des plantes...) en raison de leur stabilité de rendement supérieure. Les résultats de cette thèse permettront d'améliorer les rendements et la stabilité des cultures en mélange afin d'en faire une culture agroécologique viable.

**Title:** Breeding for mixed cropping: lessons learned from a genetic analysis of pea (*P. sativum* L.) - barley (*H. vulgare* L.) mixtures

**Keywords:** plant breeding, mixed cropping, intercropping, pea, barley, legume-based intercropping

**Abstract:** Mixed cropping (or intercropping) is the simultaneous cultivation of more than one species in the same field. Its advantages comprise higher per area yields and a higher yield stability. While the impact of different species combinations is well investigated, the potential of breeding for mixed cropping needs to be elucidated. The aim of the thesis is to explore the role of the genotype in mixed cropping and develop pathways to select genotypes with superior yielding capacity and yield stability for mixed cropping, using pea (*P. sativum* L.) and barley (*H. vulgare* L.) as a model mixed crop. In order to do so, prior knowledge is required on (a) the general (GMA) and specific mixing ability (SMA) of the genotypes; (b) the influence of a genotype on its own fraction yield (producer effect, Pr effect) and on its companion species' fraction yield (associate effect, As effect); (c) efficient experimental designs to determine GMA, Pr- and As effects of genotypes and the relative importance of GMA and SMA; (d) key-traits correlated with GMA, Pr- and As-effects for indirect selection; (e) knowledge on the efficiency of different selection strategies, i.e. direct vs. indirect selection strategies.

The thesis addresses these questions using (1) a simulation study, (2) a two-year two-location field experiment with total 32 spring pea and 8 spring barley genotypes (including genotype mixtures, GMs) in an incomplete factorial design with 64 mixtures including pure stands 3) a one-year, two-location field experiment, comparing the efficiency of two indirect selection strategies.

The simulation results suggest that a full factorial design provides only marginally better estimates for GMA and SMA variances (and individual genotypic estimates) compared to an incomplete factorial of the same dimensions but omitting  $\frac{3}{4}$  of all possible combinations. Thus an incomplete factorial allows to test the same number of genotypes with less resources.

SMA variance in pea-barley mixtures was zero or close to zero, while GMA variance of pea was of medium or small size. Both pea and barley genotypes were subject to G x E interaction with pea x year interactions and barley x location interactions being predominant. Leafy pea genotypes showed low stability for GMA and Pr effects, while pea genotype mixtures (GMs) showed high stability for GMA and Pr effects. GMA in both, pea and barley, was positively correlated with Pr effects but uncorrelated with As effects, suggesting scope for yield improvements by selecting for high Pr effects in both species. Pea pure stand yields of genotypes were correlated with their total mixture yields and fraction yields (for semi-leafless genotypes). Four key-traits for mixture performance of pea were identified: early vigor, biomass at onset of flowering and onset of flowering and stipule length. These were used for the comparison of indirect selection strategies (pure stand vs. key trait selection) with direct selection (GMA) of pea for total mixture yield. Here, pure stand yield was more efficient than key-trait selection (106% versus 64% of the efficiency of direct selection). Thus, for breeding spring pea in mixture with spring barley in Switzerland I recommend (1) (for semi-leafless types) to use available pure stand yield information for planning crossings (or for farmers to choose mixture components) (2) to select in early generations based on the key-traits presented here (3) to use a single, representative barley tester, as SMA can be neglected (4) to select genotypes with a high Pr effect in order to improve GMA (5) for breeders and farmers alike: to explore heterogeneous material like genotype mixtures with a large variability of morphological traits (especially leafiness and plant length) due to their superior yield stability. The results of this thesis help to further improve yields and yield stability of mixed crops and they support the development of mixed cropping as a viable agroecological farming practice.

*For Bruno.*

## List of abbreviations

GMA	General mixing ability
SMA	Specific mixing ability
GM	Genotype mixture
As	Associate (effect)
Pr	Producer (effect)
BIF	Biological interaction function

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# 1. General introduction

This manuscript discusses breeding for mixed cropping and uses barley and pea in organic agriculture as a model system. It develops a methodology to select genotypes in legume-based mixtures more efficiently and presents empirical data based on three years of field trials. This work focused mainly on pea as a focal species for genetic improvement in this context. Through this work it contributes to the growing body of knowledge on mixed cropping systems.

## 1.1. Breeding for mixed cropping

Breeding well-adapted cultivars for mixed cropping has been identified as one key component to boost the performance of mixed cropping and thus contribute to the attractiveness of this system to farmers. In the following subchapter, an overview on the current state of knowledge in the field of breeding for mixed cropping is given. The overview over breeding for mixed cropping in this section will mainly focus on annual legume-based mixed cropping, knowingly that a vast amount of literature has been published on herbaceous perennial legume-based mixed crops (for review Annicchiarico *et al.*, 2019). Further, I will focus on bi-specific mixtures since they are currently the most commonly used setup. After a short introduction into the main concepts in breeding for mixed cropping, which will also be used throughout the manuscript, a brief overview on the (relatively scarce) scientific literature on original research and experimentation in breeding for mixed cropping is given. However, valuable conceptual and empirical work has been done in the field of breeding for cultivar mixtures (Forst, 2018; Forst *et al.*, 2019) and many of the concepts developed there were applied to the research done in this thesis. In this introduction, a focus will be laid on pea and barley since this is the combination that has been used in the empirical part of this study as a model mixed crop.

### 1.1.1. The concept of GMA and SMA and their implications for breeding

The concept of general and specific mixing ability, abbreviated GMA and SMA, respectively, has been introduced for the first time by Federer in his pioneering work 'Statistical Design and Analysis for Intercropping Experiments' (Federer, 1993). It represents an adoption of the concepts of general and specific combining ability that have previously been defined in the context of hybrid breeding by Griffing (1956). The GMA and SMA concept have currently been applied and discussed extensively in the context of cultivar mixtures in wheat (Forst *et al.*, 2019). GMA describes the general – positive or negative – effect of a genotype on total mixture yield compared to the overall mean of total mixture yields. SMA describes the specific deviation of a pair (in bi-specific mixtures) of genotypes from their expected mixture yield (i.e. an interaction effect). I deviate here in my definition of GMA with regards to Federer (1993; who has set GMA in relation to pure-stand yields), by setting GMA and SMA in relation to the overall mean of mixture treatments. This is being done to create equivalence to the concepts used in hybrid-breeding, namely general and specific combining ability, GCA and SCA, respectively, that refer equally to the overall mean of treatments.

A simple mathematical expression of GMA and SMA can be found in equation (1):

$$y_{ijk} = \mu + G_{A_i} + G_{B_j} + S_{ij} + E_{ijk} \quad (1)$$

With  $y_{ijk}$  the total mixture yield of the k-th repetition of the mixture of genotype i of species A and genotype j of species B,  $\mu$  the grand mean of mixture yields,  $G_{A_i}$  the GMA of genotype i of species A,  $G_{B_j}$  the GMA of genotype j of species B,  $S_{ij}$  their SMA and  $E_{ijk}$  the error term. Equation (1) describes thus the bi-specific case. Methodological implications for higher order mixtures, including three or more components, have been discussed elsewhere (Federer, 1993; Forst, 2018).

The determination of the ratio of variance that is caused by GMA and SMA in mixture yields as well as their absolute values of variance are essential to determine a breeding strategy for mixed cropping (Annicchiarico *et al.*, 2019; Forst *et al.*, 2019; Hoppe, 2016). A predominance of GMA over SMA allows all genotypes of both species to be combined without deviating effects to be expected. This largely simplifies breeding efforts and subsequent seed marketing and handling by farmers. In breeding, after a test for GMA, specific combinations of genotypes do not have to be verified any more (test for SMA) and thus reduces experimental effort. In seed marketing this creates flexibility for retailers as they can combine seeds of both species by their preference (and known GMA). Finally, farmers can combine genotypes of both species based on their GMA without having to fear unexpected behavior by the choice they have made. The absolute size GMA determines the genetic leverage breeders have to influence mixture yield by means of breeding.

### 1.1.2. The concept of producer (Pr) and associate (As) effects

GMA and SMA both require information on total mixture yield. If separated yield data of both species is available ('fraction yields') the relative effects of the genotypes of each species on these fraction yields can be investigated. The producer ('Pr') effect of a genotype is defined as the effect a genotype has on its own fraction yield in a mixture, relative to the overall mean of the fraction yields of its species. The associate ('As') effect of a genotype is defined as the effect of this genotype on the fraction yield of its companion species, relative to the overall mean of the fraction yields of the companion species. Pr effects have also been called 'direct' effects in literature (Annicchiarico *et al.*, 2019; Forst, 2018; Gallais, 1976), while As effects have correspondence to the concept of 'indirect interspecific genetic effects' (IIGEs; Griffing, 1967). Pr and As effects can be of help to understand genotypes' impact on mixture ratio as well as the impact of traits on the biological interactions within a mixture (see chapter 1, p. 39).

The simplest representation of the Pr and As effects of two species in a mixed crop is expressed in equations (2) and (3):

$$y_{A_{ijk}} = \mu_A + P_{A_i} + A_{B_j} + S_{A_{ij}} + E_{A_{ijk}} \quad (2)$$

$$y_{B_{ijk}} = \mu_B + A_{A_i} + P_{B_j} + S_{B_{ij}} + E_{B_{ijk}} \quad (3)$$

With  $y_{A_{ijk}}$  the fraction yield of species A in the k-th repetition of the mixture of genotype i of species A and genotype j of species B,  $\mu_A$  the grand mean of fraction yields of species A,  $P_{A_i}$  the Pr effect of genotype i of species A,  $A_{B_j}$  the As effect of genotype j of species B on fraction yield of species A,  $S_{A_{ij}}$  the SMA effect on fraction yield of species A and  $E_{A_{ijk}}$  the error term. All parameters apply accordingly to the case of fraction yields of species B in equation (3).

### 1.1.3. Legume based mixtures

Several recent doctoral theses in Germany have in the last years been dedicated to the subject of breeding for mixed cropping. The theses of Hoppe (2016) and Starke (2018) both deal with breeding for mixed cropping of corn (*Z. mays* L.) and common beans (*Ph. vulgaris* L.) or scarlet runner beans (*Ph. coccineus* L.) for silage or biogas use, with a focus on corn in Hoppe's dissertation and on beans in Starke's. Siebrecht-Schöll (2019) investigated combinations of winter-wheat (*T. aestivum* L.) and faba bean (*V. faba* L.), while focusing on both species. A short comparison of the three experimental setups is given in Table 1.

**Table 1.** Overview on the experimental setups of Hoppe (2016), Starke (2018) and Siebrecht-Schöll (2019).

Author	Years	Legume species	Number of genotypes	Cereal species	Number of genotypes	Number of sites per year
Hoppe	2012/2013/2014	Common bean	1/3/1	Corn	160/40/25	3/3/3
Starke	2015/2016/2017	Common bean and scarlet runner bean	12/4/10	Corn	8/8/3	2/1/3
Siebrecht-Schöll	2014/2015 - 2016/2017	Winter faba bean	8	Winter wheat	3	2/2/2

While in the case of corn and beans, GMA of genotypes was significant, no significant effect of SMA was detected. In the case of winter faba bean and winter wheat, however, significant interactions between the genotypes of the two species (SMA) was detected. Fraction yields and thus Pr and As effects were not analyzed in these three theses and this thesis here is the first one to investigate and discuss Pr and As effects based on empirical data from a mixed cropping experiment with a large size of genotypes and based on a simulation study.



#### 1.1.4. Legume based mixtures: pea and barley

While the combination of pea and barley has been investigated exhaustively, notably by Steen-Jensen (Hauggaard-Nielsen *et al.*, 2001, 2001, 2006; Jensen, 1996a; Johansen and Jensen, 1996), experimental results where several genotypes for this specific combination have been tested is scarce. The following table gives an overview on existing literature in which two or more pea genotypes are used in combination with one or more companion crop.

**Table 2.** Overview of literature with two or more levels in the factor pea genotype in combination with a companion crop.

<b>Authors</b>	<b>Year of publication</b>	<b>Legume species</b>	<b>Number of genotypes</b>	<b>Companion species</b>	<b>Number of genotypes</b>
Hauggaard-Nielsen and Jensen	2001	spring pea	6	barley	5
Urbatzka <i>et al.</i>	2011	winter and spring pea	8 and 1	wheat	1
Annicciarico <i>et al.</i>	2013	spring pea	3	barley + triticale	1 each
Darras <i>et al.</i>	2015	spring pea	4	barley	1
Jacob <i>et al.</i>	2016	spring pea	14	wheat + rapeseed	1 each

#### 1.1.5. Direct vs. indirect selection: GMA vs. pure stand vs. trait data vs. marker assisted selection

Today, grain legumes are mostly bred and grown in pure stand condition. Thus, the most yield information available on genotypes is based on pure stand cultivation. The question to what extent pure-stand yield performance is correlated with mixed-stand yield performance is thus one of direct usefulness. Furthermore, if breeding for mixed cropping became more popular in the nearer future, dual breeding programs, would have to be developed. Based on the extent of the predictive capacity of pure stand yields, either no dedicated breeding programs (high correlation) or completely independent dedicated breeding programs have to be set up (low correlation) or something in between (medium correlation). In any case, the correlation of mixture yield and pure stand yield is an important parameter to know in breeding for mixed cropping. Testing large numbers of genotypes for their yield performance is expensive, irrespective of the goal, mixed- or pure-stand performance, since genotypes have to be grown and harvested in multi-year, multi-site plot trials, harvests be cleaned and weighed and processed. In any case, indirect selection by means of less expensive measurements (e.g. trait scorings, field measurements, automated phenotyping etc.) or by marker assisted selection (MAS) is a favorable strategy in plant breeding if applicable (Babar *et al.*, 2007; Cooper *et al.*, 1997; Ziyomo and Bernardo, 2013). If the mixture performance is governed by the trait-expressions of so called 'key-traits' and if these traits have high heritability, early selection of well performing genotypes is possible in the nursery or by MAS as a first selection step, and the number of genotypes can be narrowed down, less genotypes

have to be tested (saving costs) and/or the set of genotypes that is being tested is more meaningful (stronger gain of selection).

For pea, results from FSPM modeling studies have indicated that potential key-trait for performance in mixture yield might be a fast development of a considerable LAI at early stages of development, i.e. early vigor, and plant height at the onset of interspecific competition (Barillot *et al.*, 2014). Leafiness (semi-leafless or leafy plants) has also been suggested to be of importance for the performance of mixed cropping, especially under water stress (Armstrong *et al.*, 1994).

#### 1.1.6. The role of Intra-species diversity (heterogeneous genotypes)

For intraspecific diversity, represented by genotype mixtures (abbreviated in this thesis as GMs; such as cultivar mixtures or multi-line mixtures) or populations, such as composite cross populations, a long scientific record of comparisons to pure lines exist (Borg *et al.*, 2018; Reiss and Drinkwater, 2018). In the context of mixed cropping, for perennial herbaceous mixed crops, also an extensive amount of research has been conducted (for review Annicchiarico *et al.*, 2019). As a synopsis of the results of this cited literature, it can be said that intraspecific diversification tended to lead towards slightly higher yields and yield stability. For mixed cropping of annual grain legumes, the scientific record is particularly thin in terms of effects of intraspecific diversity. A literature search including the keywords 'multiline', 'multi cultivar', 'multi genotype', 'mixed cropping' and 'intercropping' did reveal two articles dealing with such an experimental setup. The first study was investigating six pea genotypes and their six possible binary mixtures together with barley (Darras *et al.*, 2015) for potential yield advantages of the GMs. The findings of this study were not consistent over experimental environments, however, and only yield advantage over pure stands was considered. Furthermore, GMs were not compared to homogeneous genotypes for their stability. The second study (Carlsson *et al.*, 2017), used two different pea GMs, consisting of two genotypes each, the first one combining a semi-leafless type (cv. Partner) with a leafy type (cv. Dukat), the second, using two semi-leafless types (cv. Dove and cv. Balltrap). In this second study, the standard error (as a measure of yield instability) did not seem to be smaller for GMs as compared to single cultivars for pure stand yields, possibly due to strong lodging of one of the two components (cv. Dukat). However, in mixed stand with wheat, standard errors of pea fraction yields were considerably smaller in the GM consisting of Balltrap-Dove as well as for one comparison (with Dukat) of the GM consisting of Dukat-Partner (of equal size for the other, Partner) when compared to the standard errors of the fraction yields of the single cultivars, giving an indication of a potentially stabilizing effect for pea fraction yields of GMs.

## 1.2. Wider context and motivation of the work

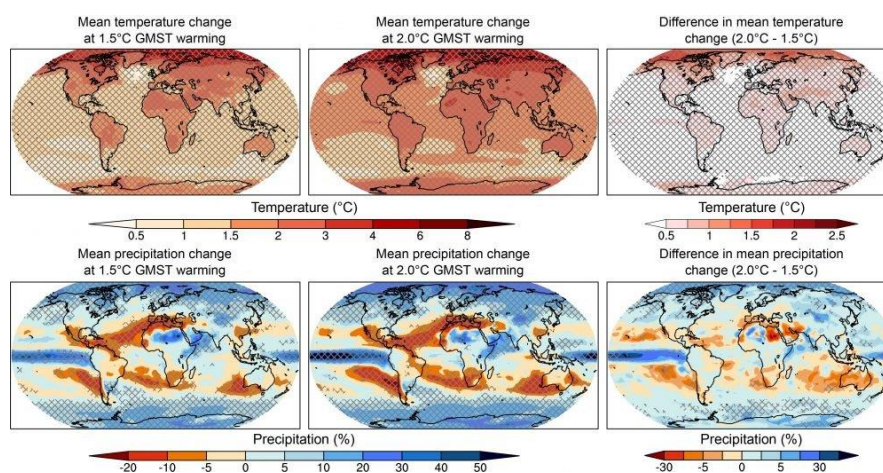
In order to understand the broader context, motivations, chances and limitations of the research presented in this study, a brief overview on the general background of the work is given. An introduction on global challenges that humanity in general and agriculture in specific are facing in the 21<sup>st</sup> century is given, followed by an introduction on mixed cropping and why it can be seen as a potential tool to mitigate some aspects of the challenges.

### 1.2.1. Challenges for agricultural production at the global scale

The global biosphere is facing major challenges in the 21<sup>st</sup> century which are intimately inter-linked: global warming, steep growth of the human population and dramatic erosion of biodiversity and the linked loss of ecosystem services, desertification and resource depletion and deterioration, including soil erosion (Aguilera *et al.*, 2020; Butchart *et al.*, 2010; Dirzo and Raven, 2003; Godfray *et al.*, 2010; McKee *et al.*, 2004; Rahmstorf and Coumou, 2011; Sheikh and Soomro, 2006). Two of these challenges, global warming and human population growth, will be presented in further detail.

#### 1.2.1.1. Global warming

If global green-house-gas emissions are going to be reduced to net zero by the year 2050, the Intergovernmental Panel for Climate Change (IPCC) projects a global temperature change of +1.5°C (as compared to the baseline pre-industrial temperature average of 1850-1900; IPCC, 2019). As a consequence, governments of 196 nations have agreed to take measures to limit global warming to +2.0°C by 2050, and preferably to +1.5°C, under the legally binding framework of the Paris climate agreement (United Nations, 2015). Different regional patterns of temperature and precipitation change are projected, depending on if a 1.5 or 2.0°C goal is going to be reached (Fig. 1). Notably, the temperatures of the +1.5°C or +2.0°C scenarios refer to *global* mean surface-temperature rises, including water surfaces such as oceans. Water surfaces are not heating up to the same extent as land areas. Thus, a change of +1.5°C will result in an actual temperature rise, e.g. in Europe between +2.5°C in maritime climates (France, The Netherlands) until up to +3.5°C in more continental or southern regions such as Romania, or in semi-continental regions in the Mediterranean, such as the center of Spain (Bindi and Olesen, 2011; Christensen *et al.*, 2007). Climate change will not only lead to higher temperatures and different regional precipitation patterns but also climatic extremes, most notably heatwaves and precipitation-extremes (Coumou and Rahmstorf, 2012; Rahmstorf and Coumou, 2011). Global warming is projected to “lead to a century of increasing climate variability and change that will be unprecedented in the history of human settlement” (Salinger, 2005). This climate variability presents unpredictable circumstances for agricultural production, which creates a need for production methods that can deal with such circumstances.



**Fig. 1.** Regional patterns of warming and precipitation change, depending on a 1.5° or 2.0°C global warming scenario by 2050. Figure from (Hoegh-Guldberg *et al.*, 2019).

### 1.2.1.2. Human population growth

The current (2019) global human population is 7.7 billion people and it is projected to increase towards 8.5 billion in 2030 and 9.7 billion by 2050 (United Nations *et al.*, 2019). While some projections assume a continuous growth until the end of the century, with a projected population-size of 10.9 billion people by the year 2100 (Ibid.), others assume a plateau around 2064 with 9.7 billion people and a decline towards 8.8 billion people by the end of the century (Vollset *et al.*, 2020). Major changes will take place in terms of regional growths and declines, with regional population growths in Sub-Saharan Africa, growing from around 1.0 billion today towards 3.0 billion by 2100. By 2100 India is projected to be the most populated country, with 1.1 billion people, followed by Nigeria with 0.7 billion people. The Chinese population is forecast to decline by about a half from today 1.4 billion to 0.7 billion, then only being the 3<sup>rd</sup> most populated country (Ibid.). Along the way, food patterns are likely to change, since global per capita income is projected to be 1.8 times higher than today (Alexandratos, 2012). If this will lead to changed consumption patterns, including to a higher consumption of livestock-based food, will depend on food prices which might also rise due to the competition of bio-fuel-production for agricultural lands (Ibid.). The relative pace at which agriculture has to attain yield improvements is said to be lower in the future than in the past, since from 1970-2010 world population grew by 3.2 billion people and is projected to grow by less, namely by 2.3 billion between 2010 and 2050. This forecast holds true even then, if more livestock-based food consumption is assumed (Alexandratos, 2012).

### 1.2.1.3. Suggestions to meet the challenges

As shown in the previous paragraphs, agriculture is facing a situation where yields have to be steadily increased in the upcoming decades to feed the growing world population, while at the same time environmental impacts, especially in terms of green-house-gas emissions, must be reduced. The current strategies to produce stable and high yields, e.g. by the application of high amounts of mineral fertilizer, are of limited future use since they themselves are a contributor to climate change (Thompson *et al.*, 2019). Organic agriculture has been suggested as an alternative pathway, and it has been shown that this system is capable to provide more benefits in terms of ecosystem services than non-organic agriculture when the baseline of comparison is unit area. (Tuomisto *et al.*, 2012). However, its benefits diminish when performance is compared on a per-unit-produced level, due to its lower per-area productivity (Muller *et al.*, 2017). Thus approaches to achieve higher yields while maintaining the ecological benefits of organic agriculture have to be found (Ibid.). Agroecological theory strives to realize high and stable yields and reducing external inputs by employing diversification as a core principle (Wezel *et al.*, 2009). By increased diversity (on an inter-species-level and/or an intra-species level), ecosystem-services can be unlocked that reduce the need for external inputs as for example fertilizers and pesticides (Reiss, 2018). Mixed cropping has been suggested as one example for an Agroecological strategy (Duchene *et al.*, 2017).

### 1.2.2. Mixed cropping as an agroecological cultivation technique

Mixed cropping is the simultaneous cultivation of two or more crops on the same field. It is often also referred to as 'intercropping'. The two terms are synonymous and throughout the manuscript the terms 'mixed cropping' or '(species) mixtures' will be used. Especially legume/non-legume species mixtures have been proposed to achieve a higher per area production and profitability as well as higher yield stability with less or no external inputs (Bedoussac *et al.*, 2015; Raseduzzaman and Jensen, 2017; Viguier *et al.*, 2018; Wendling *et al.*, 2017). The higher productivity per area, as compared to the expected yield based on pure stand yields, is sometimes referred to as overyielding, and has been systematically investigated for the first time in the pioneering work of de Wit (1960). Well-functioning pairs of complementary species have already been identified, such as combinations of corn (*Z. mays* L.) with cowpea (*V. unguiculata* L., Ofori and Stern, 1986), corn with common bean (*Ph. vulgaris* L., Hoppe, 2016; Starke, 2018), and corn with faba bean (*V. faba* L., Li *et al.*, 2003b). Furthermore combinations with small grain cereals such as barley (*H. vulgare* L.) with pea (*P. sativum* L.; Hauggaard-Nielsen and Jensen, 2001) or wheat (*T. aestivum* L.) with faba bean (Agegnehu *et al.*, 2006; Nelson *et al.*, 2021; Siebrecht-Schöll, 2019), wheat with lentil (*L. culinaris* MEDIK., Viguier *et al.*, 2018) or wheat with pea (Urbatzka *et al.*, 2011). Mixed cropping has shown to increase per area productivity under low-input conditions and has an increased land use efficiency of about +20% as compared to pure stand cultivation (Bedoussac *et al.*, 2015). It has proven benefits in terms of yield stability under varying pedo-climatic conditions (Raseduzzaman and Jensen, 2017).

### 1.2.3. Drivers and barriers of mixed cropping

Different factors promote or hinder the adoption of mixed cropping by farmers and its acceptance in the value-chain. In the following paragraphs an overview is given on the influencing factors.

#### 1.2.3.1. Drivers

(1) *Productivity*. A higher per-area-productivity of mixed cropping is an incentive for farmers to use mixed cropping as a means to increase per-area-productivity of their fields and thus to become more profitable (Bedoussac *et al.*, 2015; Viguier *et al.*, 2018). (2) *Stability*. The higher stability of mixed crops is a bonus, even if the component ratios are variable, the total yield is much more stable compared to pure stands. This fact is appreciated, especially under the projections of more variable climatic events in the face of global warming (Raseduzzaman and Jensen, 2017). (3) *Higher grain quality and profitability*. Cereals grown in mixed cropping have shown to have higher grain quality than in pure stand due to the diluting effect of lower plant densities and thus a higher availability of soil-N per plant. This improved grain quality in combination with the possibility of the simultaneous harvest of a cash-crop like lentil has shown to make mixed cropping more profitable (Viguier *et al.*, 2018). (4) *Lower weed pressure*. Mixed crops occupy niches more effectively than pure stands and use resources that otherwise would have been utilized by weeds. The weed infestation in mixed-crops is thus lower than in pure stands (Neamatollahi *et al.*, 2013) and mixed cropping thus can help to keep the weed seed-bank in the soil low. (5) *Improved lodging resistance*. Mixed crops provide severely better lodging resistance than pure stands in many occasions and thus help to prevent harvest losses or weed infestations in lodging plots by maintaining a shading canopy until harvest (Dierauer *et al.*, 2017; Hauggaard-Nielsen and Jensen, 2001).

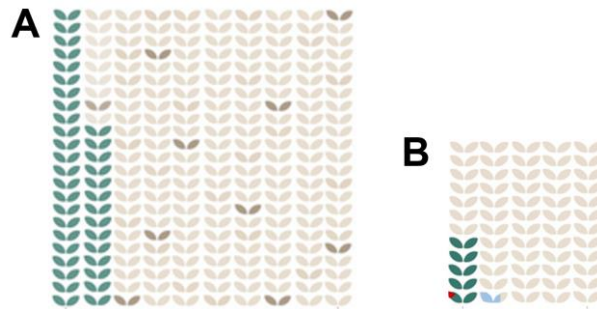
#### 1.2.3.2. Barriers

(1) *Separation*. The most important barrier for the wide adoption of mixed cropping is that harvested lots of mixed crops are often not accepted by mills and farmers cannot sell their harvested goods. Food mills have high requirements regarding the purity of their products and also feed mills are hesitant to accept mixed lots. Separation equipment for mills is an extra investment that is not profitable as long as mixed-lots make up only a marginal amount of delivered lots, therefore many mills refrain from the investment. Mills that possess the required equipment often charge an extra fee for separating the harvested goods which feeds on the farmer's benefit of harvesting more per unit area (Dierauer *et al.*, 2017). (2) *Harvest*. Harvest of mixed crops is not trivial, since often a) a compromise for the harvesting time-point between the two (or more) crops has to be found and b) also a compromise for the settings of the combine harvest has to be found (Dierauer *et al.*, 2017). (3) *Complexity*. If no prior knowledge on the phenology and maturation of species exists, the assembly of crop mixtures can be demanding and accompanied by fails. Complexity increases with the number of used species. (4) *Incompatibility of herbicides*. Many herbicides are selective for either monocotyledon or dicotyledon species, thus complicating weed control in non-organic farming systems; mostly only

a pre-sowing treatment is possible and due to legal restrictions in certain countries only herbicides that are admitted for both species are available (Strotmann, 2021). (5) *Food-safety risks due to allergens*. Many processors are wary of mixed cropping since the risk of cross-contamination with potentially allergenic by-products increases. This is especially true for foodstuff that has been grown in mixture with lupin (*L. albus* L.) or wheat (Arncken, pers. comm.). (6) *Lower N-fixation per unit area compared to legume pure stand*. The amount of nitrogen derived from atmosphere by fixation of legume plants per unit area is lower in mixed stands as compared in pure stands due to the lower plant density of legume plants and their lower fixation rate per plant (Bedoussac *et al.*, 2015). In crop rotations mixtures often compete with the pure stand cultivation of the legume plant and the farmer has to decide for either pure stand or mixed cultivation of the legume, in the latter case having to consider a lower value than legume pure stand in terms of nitrogen deposit in the soil for the subsequent fruit. (7) *Lack of well-adapted genotypes*. Several studies have shown the large impact of the genotype on the performance of mixed crops (Annicchiarico and Piano, 1994; Barillot *et al.*, 2014, 2012; Hauggaard-Nielsen and Jensen, 2001; Hoppe, 2016; Siebrecht-Schöll, 2019; Starke, 2018). While plant breeding currently focusses nearly exclusively on the breeding for pure-stand cultivation, pure-stand performance translates only partially in mixed stand performance (for review Annicchiarico *et al.*, 2019). There might thus be a lack of well-adapted genotypes for mixed cropping that currently still locks the full potential of this growing system. Breeding adapted genotypes for mixed cropping might change this situation.

#### 1.2.4. Case study: mixed cropping in Switzerland

Switzerland has a total land surface of 4.1 million ha of which 1.0 million ha are agriculturally usable (excluding pastures in the alps and Jura mountains; BLW, n.d.). Its total *arable* farmland 2018 was 272 000 ha, i.e., about one quarter (27%) of total agricultural land. The rest, three quarters, is used as grassland (Fig. 2). Of the *arable* land, 8% (21 000 ha) is organically managed. However, of the *grasslands*, 19% (139 000 ha) are organically managed. In total, 16% (160 000 ha) of all farmlands is managed organically. In conventional agriculture, 29.1% of all farmlands is used as arable land, whereas in organic agriculture it is only 13.2%.



**Fig. 2.** (A) Total agriculturally used land in Switzerland, including arable land and grassland. Organically managed land represented in green symbols (16%). Of this total surface, the total arable land is 27% (B). Organically managed areas are marked green (8% of arable land), the area of organically grown grain legumes in red (6.6% of organically managed arable area) and the area of non-organically grown grain-legumes in blue (2.2% of non-organically managed arable area). Figures from BLW (n.d.).

On 6.6% of the organically managed arable lands grain-legumes are grown, while it is only on 2.2% of the non-organically managed land (BLW, n.d.). As a result of the policy of Bio-Suisse, the biggest growers-association for organic producers in Switzerland, which had created an incentive-system for growing grain-legumes, and investments of large feed-mills in separation gear and thus their readiness to accept mixed lots of harvest, nearly 100% of all organically grown grain legumes in Switzerland are grown as mixed crops (Dierauer *et al.*, 2017). Mixed cropping thus represents a niche in the Swiss agricultural landscape, yet a very well established one.

### 1.2.5. Current fields of research in mixed cropping

Genomic selection has been suggested as a cost effective selection tool to predict the breeding value of crops in mixed stand condition (Annicchiarico *et al.*, 2019). This technique would be especially efficient if the costs of phenotyping are higher in mixed stand as compared to pure stand and if the power of the models would allow to predict mixed stand yield reliably over several companion genotypes (Ibid.). Recent simulation studies are suggesting that genomic selection is superior in terms of efficiency and selection gain compared to phenotypic selection (Bančič *et al.*, 2021).

While genomic prediction can be categorized as a so called 'trait-blind' approach, modeling of plant interactions, using Functional-structural plant modeling (FSPM), can be used to understand biotic interactions, such as competition or mutualism, within plant stands that are the result of specific traits (Barillot *et al.*, 2019, 2014). This approach simulates the growth dynamic of plant communities *in silico* and thus provides the possibility to test a multitude of hypotheses regarding the influence of traits and environmental factors on the interplay of individuals and species within a plant community without having to test these in pot or plot trials. It allows to create assembly rules for species, based on trait combinations that were tested successfully in simulations. Crop modeling can thus contribute to the establishment crop ideotypes for breeding (Donald, 1968). It



further is helpful in determining crops' impact on environment (Liu *et al.*, 2016), identifying limiting factors in crop productivity (Brisson *et al.*, 2010) and helping to minimize inputs by suggesting improved management (Bergez *et al.*, 2001; Chatelin *et al.*, 2005). Latest advances in FPSM modeling of mixed cropping include insights on nonrandom assembly of trait diversity (Louarn *et al.*, 2020).

In order to increase the area covered by mixed crops in Europe and worldwide, integrated approaches, bringing together basic research, applied research and extension is required to facilitate the knowledge transfer that is needed to reach this goal. In the following, one European research project is going to be described that was designed to facilitate the transition towards more crop-mixture based agriculture.

### 1.2.6. EU-Project ReMIX

The work of this thesis was done in the framework of the EU-Horizon-2020 project 'ReMIX – Redesigning European cropping systems based on species MIXtures'. Together with another project ('DIVERSify') the project was part of the EU call H2020-SFS-2016-2017 with the topic of 'Sustainable Food Security – Resilient and resource-efficient value'. The goals of the project were to " 1) overcome barriers to stimulate the adoption of species mixtures by farmers and in agri-food-chains, 2) unravel mechanisms of plant-plant interactions to maximize resource use efficiency, 3) determine the role of species mixtures in controlling diseases, pests and weeds and alleviating yield damages, 4) demonstrate the role of species mixtures in improving ecosystem service provision and development of resilience to biotic stress, 5) identify key traits and create novel breeding and phenotyping methods and tools adapted to species mixtures, 6) generate novel breeding material adapted to grain legume/cereal mixtures, 7) develop generic rules for assembling species for efficient cash crop production using process-based simulation models, 8) develop new management techniques to optimize species mixtures performance, 9) optimize settings and specifications for agricultural machines for harvesting and separating grains, and 10) develop a tool box, an educational serious game and technical booklets for farmers and advisors." (From: Remix full proposal, 2016). The consortium had 23 participating institutions located in thirteen European countries and China and the project duration was 48 months, starting in Mai 2017. This thesis was embedded in work package four, with the topic 'Screening, breeding and phenotyping methods for species mixtures.

After this general overview on the context of the thesis, an introduction to the underlying biological principles that guide plant-plant-interactions in mixed cropping will be given in order to understand the principal mechanisms that shape the growth dynamics in these plant communities.

### 1.3. Underlying principles for overyielding of species communities in general and in legume-based mixtures in specific

In the subsequent paragraphs, modes of plant-plant interaction are described that occur in plant communities. While for the specific interaction more precise terms from ecology have to be used [refer to chapter with BIFs], the subsequent terms are useful to describe general phenomena in plant-plant interactions. A specific focus is laid on interactions taking place in legume based mixed crops and examples from literature are given.

#### 1.3.1. Competition

Individuals in a plant community all compete for the same basic resources: light, water and soil nutrients. While aboveground competition is mainly limited to light, belowground competition is more complex, as, besides for water, plants compete for a multitude of different soil nutrients. (Casper and Jackson, 1997). Competition occurs either among genetically closely related individuals, e.g. of the same species (intraspecific competition) or among different species (interspecific competition). Important examples for plant-plant competition are the shade avoidance syndrome due to competition for light that leads to internode elongation as a competitive strategy (Ballaré *et al.*, 1991; Schmitt *et al.*, 1995); for water, different uptake efficiencies in species exist due to different root architectures (Homulle *et al.*, 2021; Zegada-Lizarazu *et al.*, 2012) that might lead to competitive differences between species; also for nutrients, differences in uptake efficiencies between different species have been reported (Föhse *et al.*, 1988; Pettersson and Jensen, 1983). For nitrogen, a big discrepancy in uptake efficiency exists between legume and non-legume plants (Annicchiarico *et al.*, 2019; Bedoussac *et al.*, 2015; Jensen, 1996a).

#### 1.3.2. Resource-use complementarity

Resource-use complementarity is one of the main drivers of overyielding in mixed cropping and is facilitated by the niche differentiation of species or genotypes (Duchene *et al.*, 2017; Hauggaard-Nielsen and Jensen, 2001; Willey, 1990). Niche differentiation causes lower interspecific competition as compared to intraspecific competition and consequently leads to more resources available to each individual as (intraspecific) competitors for this specific resource are spatially or temporally dispersed and diluted by individuals of another, complementary, species. Important cases for resource-use complementarity in legume based mixed cropping comprise light-use, soil nitrogen (N) and root space. (1) *Light*. In one example, Bedoussac and Justes (2010) have shown that irradiated light is used more efficiently with in a winter-wheat winter-pea mixed crop as compared to sole-crops due to different temporal growth dynamics of the two crops. This was due to different peaks in leaf area index leading to a longer photosynthetically active canopy area and thus resulting in a 10% higher net assimilation of the mixed crop as compared to the pure-stands, which is providing one explanation for overyielding in this crop. (2) *Nitrogen*. The resource nitrogen is used in a complementary way in mixed cropping with non-legume crops due to the unique capacity of legumes to switch their N-acquisition

strategy from taking up soil-N to fixating atmospheric N in situations where soil-N becomes limiting (Duchene *et al.*, 2017) thus alleviating interspecific competition for this key-resource. (3) *Root space*. As for root space, studies have shown that complementarities between species in terms of exploring different soil horizons are either pre-existing, such as for maize (tendency to shallow rooting) and common bean (exploring the vertical soil profile more equally; Zhang *et al.*, 2014) or are expressed under the presence of a second species, for example the deeper soil penetration of faba-bean at the presence of wheat (Bargaz *et al.*, 2015). While root space is not a resource *per se*, soil resources such as nutrients and water are better available if the soil space is used in a dispersed manner. Resource-use complementarity is reported to play a bigger role in stressful environments, where usually a higher per area efficiency of mixed crops as compared to pure-stands is attained (Bedoussac *et al.*, 2015; Reiss, 2018). This effect is attributed to the so called stress gradient hypothesis, by which a positive effect of diversity is enhanced in environments with higher stress (He *et al.*, 2013).

### 1.3.3. Host dilution

Host density by unit area is decreased in mixed crops due to the dilution by the companion species, thus decreasing the exposed host-surface per area and providing barrier effects for pathogens and pests (Boudreau, 2013). This diversity related effect is well known from experiments with multiline mixtures of wheat (Knott and Mundt, 1990). In mixed crops of pea and barley, reductions in important diseases were reported for *Microsporella pinoidea* (Fernández-Aparicio *et al.*, 2010), *Pyrenophora teres* (Kinane and Lyngkjær, 2002). Hauggaard-Nielsen *et al.* (2008) report a general disease reduction in mixed crops of barley with pea, faba bean and lupine of 20-40%.

### 1.3.4. Push-pull mechanisms

Push-pull mechanisms have been observed in mixed crops and can be exploited to increase yield and yield stability (Bhattacharyya, 2017; Hailu *et al.*, 2018). While none of these effects are (yet) known for legume-based mixed cropping in temperate climates, future applications might be arising. The most well-known system in which a push-pull mechanism has been implemented in practical farming is the mixed cropping of cereals, e.g. corn, with plants from the desmodium genus, e.g. *Desmodium uncinatum*, herbaceous legume plants that repel stemborers, e.g. *Busseola fusca* Füller (push), and napier grass, *Pennisetum purpureum* Schumach, a grass that is planted at the border rows that attracts the latter (pull; Khan *et al.*, 2014; Pickett *et al.*, 2014).

### 1.3.5. Facilitation

A further plant-plant interaction is facilitation, i.e., the positive effect on the performance a mixture component facilitated by the presence of another component. Facilitation can be subdivided in direct facilitation, i.e. by mechanisms by which one plant directly profits from a transfer of nutrients from another, and indirect facilitation, i.e. by changes of the environmental conditions towards a favorable way for the companion species (Duchene *et al.*, 2017). (1) *Direct facilitation*. Direct

facilitation occurs in the form of rhizodeposition, i.e., the release of organic or inorganic compounds by the roots of one species and its absorption by another. One important form of rhizodeposition is the release of nitrogen derived from atmosphere by fixation into the soil matrix by legumes. Soil N derived from rhizodeposition (NdfR) reaches the soil matrix by gradually decaying root components and is the major source of nitrogen for grasses in herbaceous mixed crops (Hammelehle *et al.*, 2019). For a transfer of NdfR to the cereal crop in cereal-legume annual mixed crops there is no consensus in the literature. Jensen could show a transfer from pea to barley of 19% 70 days after sowing in a pot experiment (Jensen, 1996a), probably from decaying pea root material, and Johansen and Jensen (1996) could show that the flow of N and P between pea and barley is largely dependent on the presence of an arbuscular mycorrhizal fungus, *Glomus intraradices*, that facilitates the uptake of N from decaying root material from of pea. These findings could not be confirmed (but also not rejected) in a field experiment (Jensen, 1996b). A conservative assumption should thus be that NdfR is not being transferred in annual legume-cereal mixtures but is well transferred in perennial herbaceous mixed-crops. A second direct facilitative effect involves the release of facilitative root exudates, which is observed in corn- (*Z. mays* L.) faba bean (*V. faba* L.) mixed crops. It has been shown that corn – in contrary to wheat and barley – drives the nodulation of faba bean by the exudation of flavonoids that switch on genes involved in the mediation of nodulation and auxin response, leading to a significantly increased nitrogen fixation in faba bean (Li *et al.*, 2016). (2) *Indirect facilitation*. The shading effect of legumes, suppressing weeds while not competing with the non-legume component for one of the major resources – nitrogen – can be seen as indirect facilitative effect in mixed crops. The non-legume thus profits from less competition for soil nitrogen by weeds and a much larger pool of nitrogen is thus available (on a per-plant basis) as in pure stand and if competition for light and other resources by the legume plant is moderate. More vigorous plants and higher protein contents per plant in the non-legume are thus the consequence (Bedoussac *et al.*, 2015; Bedoussac and Justes, 2010b; Viguier *et al.*, 2018). A further group of indirect facilitative effects includes the alteration of the soil chemistry in a favorable way for the companion species. Many nutrients are stability bound by chemical compounds in the soil and their availability for plant-roots are low (Hinsinger, 2001). Changes in different soil-chemical properties by plants trigger the release of important nutrients such as phosphorous, mainly, but not exclusively, by lowering the soil pH (Ibid.). A significant increase in P uptake has been reported for chickpea-wheat mixed crops, resulting in higher wheat biomass due to this effect (Li *et al.*, 2003a). A similar mechanism has also been observed in wheat-lupine mixed crops (Cu *et al.*, 2005) and maize-faba bean (Li *et al.*, 2010).

## 1.4. Background on pea and barley as a model mixed crop used in this study

Pea (*P. sativum* L.) and barley (*H. vulgare* L.) were used as a model crop in this thesis and their species particularities are going to be introduced in this subchapter.

### 1.4.1. Pea

#### 1.4.1.1. General description of the crop's history, its traits and plant types

Wild ancestors of cultivated pea were widespread in the Mediterranean (*Pisum sativum* subsp. *elatius*) and the fertile crescent (subsp. *humile*) where they co-evolved with undomesticated forms of cereals in prairies or as a weed in cultivated cereals (Schilperoord, 2018). First archeological accounts of collected wild peas were found in Israel and are 23000 years old. First accounts of cultivated forms date back to 8500-8200 B.C. in South-Syria (Zohary *et al.*, 2012). Over the Mediterranean, the cultivated form spread over Europe and in Switzerland peas were found at a site close to lake Constance and date back to 3380 B.C. (Schilperoord, 2018). The widespread use of peas in Europe are also thanks to Charlemagne, who, with his *Capitulare de villis*, written about 795 A.C., created a binding list of cultivated plants that had to be grown in the Carolingian Empire which included pea as a compulsory crop. Dried peas were a staple food in Roman times and in the Europe of the Middle Ages.

*Leaf morphology.* Three different leaf types exist in peas: leafy, semi-leafless ('afila') and leafless, the latter two miss the semi-leaves at the side branches that are transformed into additional tendrils in this case, the last type, leafless peas, in addition also miss the stipule leaves that grow otherwise pairwise at every node. While leafy types have shown higher water use efficiency and have proven more resistant against drought stress (Armstrong *et al.*, 1994), semi-leafless and leafless types show dramatic improvements in lodging resistance (Gosal and Wani, 2020) while lacking the ability to adjust leaf angles under drought stress to minimize the fraction of photosynthetically active radiation that is intercepted (Ridao *et al.*, 1996). Leafless types do not play a role in current cultivation due to their low yield capacity due to exceptionally low photosynthetic area (Ibid.). Besides these types, a plethora of other leaf morphologies in pea exist (Yaxley, 2002). Lodging in pea can cause severe yield losses (up to 74%) due to a more fungi-friendly micro-climate in the lodged canopy, hampered assimilation capacity and harvest losses. Semi-leafless types are nowadays the mainly cultivated leaf-type with 95% and 80% in Canada and the European Union, respectively (Gosal and Wani, 2020).

*Dwarfing genes.* Peas have undergone dramatic selection towards shorter genotypes to improve lodging resistance and whereas traditional cultivars were at a shoot length of about 200 cm, modern cultivars can be as short as 30 cm. This dramatic shortening of the stem is amongst others genetic factors due to a widely used mutation of a single dwarfing gene (*le-1*) with Mendelian inheritance (Gosal and Wani, 2020).

*Winter and spring type.* In a comparison of autumn sown and spring sown pea Urbatzka *et al.*,

(2011) found that the autumn sown forms of pea had higher yield potential than spring sown types (fraction yield of best winter pea was higher than yield of spring pea pure stand).

#### 1.4.1.2. Current cultivation and use

Today three types of peas of agronomic importance (1) field pea (*P. sativum* subsp. *arvense* L.) which is harvested at maturity of the hard grains (2) garden pea (*P. sativum* subsp. *sativum* convar. *medullare* L.) whose sweet-tasting seeds are harvested at dough-ripeness (3) sugar peas (*P. sativum* subsp. *sativum* convar. *axiphium* L.). The total area of cultivated field pea in 2019 (latest data) globally was 7 million ha and of all green peas, i.e., garden and sugar peas, 2.8 million ha. As a comparison: of barley 51 million ha and of wheat 216 million ha were harvested. Since field pea is the more important pea in terms of production area, in the following, values are given for this form. The main producing countries in terms of production surface of field pea are Canada (24%), the Russian Federation (17%) and China (13%) (FAOSTAT, 2021). Europe accounted for 12% of global production area with the three main producing countries France, Spain and Romania with 2.5, 2 and 1.5 % of global field pea growing surface, respectively. The main use of field pea harvests is as feed. Same as for barley, field pea has lost its long bearing as a staple food in most regions of the world and garden pea is the main form that is being consumed nowadays, mainly as a garnish, fresh or canned (Toussaint-Samat, 2009). The only dried form that is still frequently used for human consumption are split peas, which are itself an invention of the late 19<sup>th</sup> century, and which are field peas whose skin has been mechanically rubbed off (Ibid.). The trend towards meat-substitute products have opened up a new uses for dried field pea seeds for human consumption (Hayes, 2018) and products are successfully being marketed (Beyond Meat, 2021; Planted, 2021 and others).

#### 1.4.1.3. In the context of mixed cropping

Compared with other grain-legumes, most notably lentil and common bean, pea has a much lesser requirement for a supporting companion crop to avoid lodging. This is especially true for semi-leafless types which have a relatively good lodging tolerance, but less for leafy types. Nonetheless is a combination with a nitrogen-dependent companion crop advisable, especially in organic farming since the free niche space in pea is otherwise occupied by weeds.

### 1.4.2. Barley

#### 1.4.2.1. General description of the crop's history, its traits and plant types

While some research points to the fact that the botanical origin of barley (*Hordeum vulgare* L.) might lay in Southeast-Asia (China, Tibet or Nepal; Tadele, 2018), it's exact center of origin is still unknown. Barley got domesticated in the so-called Fertile Crescent about 8000 BC (Ibid.). Barley enabled the Neolithic revolution, together with einkorn (*T. monococcum* L.) and emmer-wheat (*T. turgidum* L.; Zohary *et al.*, 2012). For a long time, barley was the 'rice of the west' and a staple food in western Europe, consumed as a mesh or soup. It was only in the 17<sup>th</sup> century that it lost its state due to the gaining popularity of potato and a more bread based diet (Schilperoord, 2013). *Phenology.* Barley has a much earlier flowering time than cereals of the *Triticum* genus. This fact

allows barley to be cultivated at marginal agricultural regions where due to climate (the temperature sum during a growing season would be too low to grow wheat for example) no other cereals can be grown, notably at higher elevations and mountainous regions (Schilperoord, 2013). Under Central-European temperate climatic conditions, this early phenology causes that barley can be harvested usually two to four weeks before wheat. The earliness of the crop also is a key-requirement to grow it as a mixed crop with spring sown pea. While spring wheat or oats would still be at dough stage, barley is mostly at good synch with pea maturity and thus a well-suited partner. *Two-, and six-row barley.* In two-row barley only the middle of three branches of a floret is fertile, while in six-row barley all three florets are fertile, which makes the ear appear to have two or six kernel-rows, respectively. The number of rows is controlled by a single gene and six-row barleys are the phenotype of the homozygous, recessive genotype (Schilperoord, 2013). Due to its superior grain uniformity, two row barley is mostly used for malting purposes (Tadele, 2018). *Spring and winter barley.* Spring barley is mostly of the two-row form and used for malting purposes. Winter barley is mostly of the six-row form and used for feed. *Hulled and hull-less barley.* Two forms of barley grains exist: hulled and hull-less. In the hulled form, the two covering glumes, the lemma and the palea, are tightly attached to the grain. Same as the number of rows, this trait is also controlled by a single gene, and homozygous, recessive genotypes are hull-less (Schilperoord, 2013). *Awns.* In contrast to wheat (*T. aestivum* L.), where awned and unawned types exist, barley is always awned. Its long awns can be double the length of the ear and still contribute to assimilation when the leaf surface is already dried out in late stages of maturation or due to fungal diseases (Schilperoord, 2013).

#### 1.4.2.2. Current cultivation and use

The main use of barley for human food-consumption has diminished and in terms of quantity, its use as feed is the major utilization and 75-80% of the global barley harvest are used as an animal feed. Economically however, malting is the most important usage which accounts for 20-25% of its harvest. Only 2-5% serve as food which is consumed in certain areas of the world, notably in the Andean and Himalayan regions and in Ethiopia (Tadele, 2018). In some regions of Europe, notably on the Canary Islands, a mesh, based on roasted barley-flour 'gofio' is still prepared as a side-dish. In Switzerland barley-soup, using pearled barley, made from the hulled form, is still a local specialty in south-east of the country. Europe is the largest producer of barley, followed by Asia (Ibid.). The total area of cultivated barley in 2019 (latest data) was 51 million ha. As a comparison: of pea 7 million ha and of wheat 216 million ha were harvested. The main producing countries in terms of production surface of barley are Europe (24%), the Russian Federation (17%) and Australia (9%) (FAOSTAT, 2021). Within Europe, Spain is the most important barley producing country, followed by Germany and France with 5, 4 and 3% of the global production surface, respectively.

#### 1.4.2.3. In the context of mixed cropping

Due to its early maturation, barley can be seen as an ideal mixing partner for spring pea. A further advantage is, that both components, pea and barley, are used as animal feed and thus separation does not have to fulfill the same strict requirements as for goods destined for human consumption. While desirable for bread and pasta wheat (Bedoussac and Justes, 2010b; Viguier *et al.*, 2018), a high protein content in two-row spring barley, whose main use is for malting, is not desired, as high protein negatively influences brewing and distilling processes and recommendation for malting barley for distillation is a protein content of <8.8% (Hackett and Park, n.d.). Harvested lots from legume based mixed cropping thus cannot be sold to the high prices usually attained for this crop.

### 1.5. Research objectives

In this manuscript, the following research objectives will be addressed:

- (1) To compare suitable experimental designs for breeding experiments for mixed cropping;
- (2) To elucidate the absolute and relative sizes of GMA and SMA variance in a pea-barley mixed crop with genotypes that are representative of current central-European breeding material;
- (3) To investigate the correlation between pure stand yields in pea and total mixture yields as well as pea fraction yields in mixture;
- (4) To explore the impact of genotypes on mixture performance, e.g., to determine the GMA, Pr and As effects of genotypes and their stability across environments;
- (5) To investigate the role of key traits, based on prior knowledge (early vigor, plant height, stipule area, leafiness, and biomass) or to mine new traits as potential predictors for GMA.
- (6) To propose adapted breeding strategies based on the previous results.

The following section will first describe the overall experimental setup. Afterwards, three thematic chapters follow. A first one presenting a simulation study, comparing several experimental designs for their efficiency to estimate GMA and SMA variances, followed by a presentation of concepts that are useful in the context of breeding for mixed cropping. A second one applying these theoretical considerations on empirical data of a two-year, two-site field experiment, in which empirical parameters are presented and discussed (GMA, SMA and their variances, Pr- and As effects, stability measures for these parameters, LER, genotypic effects on ratios, key-trait-measurements and a comparison of pure stand and mixed stand behavior of genotypes). Finally, the efficiency of indirect selection based on key-traits and pure stands data is being compared.



## 2. Experimental setup

The research work was divided into three thematic chapters. The first chapter provides the theoretical and methodological groundwork for the thesis, including (i) a simulation study comparing different experimental designs for their capacity to provide estimates for GMA, SMA and genotypic breeding values (ii) the development of the concept of Pr and As effects for mixed cropping research and –experiments, as well as (iii) establishing a concept to classify traits for their biological interaction function (BIF). The second chapter comprised a multi-year, multi-site and multi-genotype mixed cropping field-experiment (experiment 1 in the following) that was conducted in order to find empirical values for the most important parameters in breeding for mixed cropping: (i) size of GMA and SMA variance, (ii) yield and key-trait measurement values and their heritabilities and (iii) individual genotypic breeding values. The third chapter represented a one-year multi-site experiment (experiment 2) that was piloted in order to test one direct and two indirect selection strategies for selecting superior genotypes for mixed cropping, employing the experimental parameters (GMA, pure stand yield and key-trait measurements) gained from the second chapter.

The following chapter will focus on the description of the experimental conditions of the two empirical chapters, e.g., the soil and climate, the experimental design used, the genotypes. The description of the first (theoretical) cluster will be provided in the corresponding chapter (see chapter 3). For more specific descriptions of the methodology applied in every chapter, please refer to the corresponding materials and methods sections.

### 2.1. Experiment 1 (2018 and 2019)

#### 2.1.1. Plant material

Twenty-three pea genotypes and four pea genotype mixtures (GMs) as well as seven barley genotypes and one barley GM were used in the experiment. As for pea, competitive ability towards a companion crop is strongly determined by competition for light around flowering time (Bedoussac and Justes, 2010a) and soil N at early growth stages (Corre-Hellou *et al.*, 2006a) and thus data on plant length, leafiness and early vigor was collected from breeders and national seed authorities prior to sowing (see Table 3, following page).

**Table 3.** The twenty-three pea genotypes used in the experiment plus the four genotype mixtures and the five discarded genotypes, P15, P16, P17, P23 and P30. Trait data gathered prior to sowing is indicated by an 'x'. For certain cultivars no prior information on the trait could be gathered and '(x)' represents the empirical value. Genotypes marked with both an 'o' and an '(x)' proved to be of a different trait value in the experiment than indicated by prior data, with '(x)' being the measured trait value. Plants-length-categories (small, medium, or long) can be seen by the 'x' or '(x)' entry in the corresponding column for stem length. Genotype mixtures (GMs) for stem length (GM-long and GM-short) were purely based on prior data, i.e., contain all genotypes with an 'x' or an 'o'.

Genotype	Pea code	Stem length			Semi-leaves		Early vigor			Breeding program (country)
		short	medium	long	present	absent	low	medium	high	
SG-L 7647	P01			x		x		x		Selgen (CZ)
Impuls	P02			x		x			x	Selgen (CZ)
Astronauta	P03	x				x		(x)		Saaten Union (NPZ Lembke) (D)
Navarro	P05	o	(x)			x		(x)	o	Saaten Union (NPZ Lembke) (D)
Gambit	P06			x		x			x	Selgen (CZ)
Angelus	P07	(x)		o		x	x			Lemarie Deffontaines (F)
Salamanca	P08			(x)		x		(x)	o	Saaten Union (NPZ Lembke) (D)
Rocket	P10		(x)			x	(x)			Lemarie Deffontaines (F)
Karpate	P11	x				x		(x)		KWS Momont (F)
Kayanne	P12	o	(x)			x	(x)			KWS Momont (F)
Mytic	P13	x				x	(x)			Agri Obtentions (F)
Protecta	P14			(x)	x				(x)	Selgen (CZ)
Mehis	P15				x			(x)		Estonian Crop Research Institute (EE)
Tarchalska	P16	o	(x)			x			(x)	Danko (PL)
Bluetooth	P17	x				x	x			RAGT (F)
Alvesta	P18	x				x		(x)	o	KWS (D)
Bockros	P19	o	(x)			x			x	Selgen (CZ)
Volt	P20		x			x	o	(x)		RAGT (F)
Biathlon	P21	x				x	(x)			Florimont Desprez (F)
Tip	P22		(x)	o		x			x	Selgen (CZ)
Vitra	P23			x	x				(x)	AREI (LV)
Peps	P24			(x)		x		(x)	o	Selgen (CZ)
Karioka	P25	o	(x)			x		(x)		KWS Momont (F)
Milwa	P27	x				x	(x)			SMOLICE (Hodowla Roslyn Smolice) (PL)
Florida	P28			x	x				(x)	Saaten Union (NPZ Lembke) (D)
Natura	P29	(x)			x			(x)		Selgen (CZ)
Audit	P30		(x)	o		x		(x)	o	Limagrain (F)
Starter	P31	x				x	(x)			Saaten Union (NPZ Lembke) (D)
GM-short	P09									GM containing all short-stemmed genotypes
GM-long	P26									GM containing all long-stemmed genotypes
GM-semi-leafless	P04									GM containing all semi-leafless genotypes
GM-leafy	P32									GM containing all leafy genotypes

Assuming the same importance of these competition aspects for the dynamics within mixture for barley, data on traits associated with early growth habit and competitiveness for light were assembled prior to sowing. For barley, stem-length-, flag-leaf size-, early height-, planophile or erectophile growth- and tillering capacity-data was collected (see Table 4).

**Table 4.** The seven two-row spring barley genotypes and the genotype mixture (GM) used in the experiment. A competition score was given prior to the genotype choice based on data from breeders and national seed authorities on five morphological traits that convey either high competitiveness (left side of the respective trait-column) or low competitiveness (right side of the trait column). For this score, trait-values were summed up and added with +5 in order to receive a value between one and ten. Where no data was available, a value of zero was assumed.

Cultivar	Barley code	Competition score (1=low, 9=high)	Stem length		Flag leaf size		Planophile/erectophile		Tillering capacity		Breeding program (country)
			long	short	large	small	planophile	erectophile	high	low	
Grandiosa (DZB0913c)	B1	9	3		1		1			-1	Cultivari (D)
Eunova	B2	8	2				1				Probsdorfer Saatucht (AT)
GM-barley	B3	6									multi-cultivar mixture
Zeppelin	B4	1		-2		-1		-1			Sejet (DK)
KWS Atrika	B5	5	0								KWS (D)
Propino	B6	4	0			-1					Syngenta (D)
Rubaszek	B7	5	0								Smolice (PL)
KWS Irina	B8	6		-2					3		KWS (D)

Pea genotypes were categorized for each trait and in the choice of the genotypes for the experiment, a balanced amount of all trait-categories in order to represent the whole range of these traits in current European spring-pea material. For pea, in the choice of the genotypes a wide and balanced and broad range of trait values for plant-length, leafiness and early vigor were tried to achieve in selecting the genotypes for the experiment as these were seen as candidates for key-traits. For barley, a competition score was given to each cultivar prior to the cultivar choice in order to display a representative range of competitiveness in current European two-row barley. All genotypes are abbreviated with a number code starting with "P" for peas and with "B" for barleys throughout the manuscript. All used pea genotypes were released cultivars under central European conditions for grain usage. The only exception was P28, which is advertised as a dual-use cultivar for grain and silage use. All barley genotypes represented current cultivars released under central European conditions and destined at feed usage (B2 and B7) or malting usage (all others). Based on breeders' data, during designing the experiment, genotype-mixtures (GMs) were created, one mixing all short genotypes ('GM-short', containing 13 genotypes) and another one, mixing all long genotypes ('GM-long', containing seven genotypes). Another two GMs were created, one mixing all semi-leafless genotypes ('GM-semi-leafless', containing 23 genotypes) and one mixing all leafy genotypes ('GM-leafy', containing

five genotypes of which one, P15, was excluded from the main analysis, see below). These four GMs are part of the 27 genotypes used and will be referred to also as genotypes in this manuscript. Idem for barley, one GM ('GM-barley'), consisting of all seven barley genotypes, was created, and will also be referred to as genotype in this manuscript. Data of different breeding companies on plant length was not consistent with our field observations, thus, pea genotypes were characterized by us for their plant length at stretching (by measurements in the experiment) and the length categories used throughout the manuscript ('short', 'medium', 'long') refer to this a posteriori categorization. Out of the thirty-two pea genotypes initially chosen, two had to be excluded due to their too late maturity (P15 and P23) and three others due to extremely poor field emergence in the first experimental year (P16, P17 and P30) resulting in twenty-seven pea genotypes finally used for analysis.

### 2.1.2. Sites, soils, and trial management

The experiments were carried out over two subsequent years (2018, 2019), at two organically managed locations in Switzerland, Fislisbach ('Fis') and Uster ('Ust'). Sowing took place on March 26 in 2018 (Fis and Ust) and on March 23 (Fis) and 24 in 2019 (Ust). Harvest was done on July 11 (Fis) and 18 in 2018 (Ust) and on July 18 (Fis) and 20 in 2019 (Ust). Pure stands were sown with sowing densities of 100 viable grains/m<sup>2</sup> for peas and 400 viable grains/m<sup>2</sup> for barley. Mixtures were sown as an additive design in a ratio of 80% of pure stand density for pea (resulting in 80 grains/m<sup>2</sup>; 100 grains/m<sup>2</sup> in pure stands) and of 40% of pure stand density for barley (resulting in 160 grains/m<sup>2</sup>; 400 grains/m<sup>2</sup> in pure stands), totaling 120% sowing density and following local recommendations (Dierauer *et al.*, 2017). Pea and barley were blended within the rows. Plots were sized 1.5m wide x 4.7 m long (7.1 m<sup>2</sup>) with either seven rows with 19 cm spacing each (Fislisbach) or six rows with 21cm spacing each in between. Mineral soil nitrogen (NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>) at sowing time ranged from 35 kg/ha (Fis 2019) to 117.8 kg/ha (Ust 2019). No supplementary fertilization or mechanic weed control was applied. See Fig. A3 for climate data, Table A4 for soil data on the experimental environments and Fig. 3 for visual impressions of the four experimental environments.



**Fig. 3.** The four experimental environments, clockwise, starting from top left: Fislisbach 2018, Fislisbach 2019, Uster 2019 and Uster 2018.

### 2.1.3. Experimental design

An incomplete factorial design was used that combined every pea cultivar with two barley cultivars and every barley cultivar combined with eight pea cultivars. The design was randomized as follows: four 8x8 Latin squares were created with different randomizations each and dummy entries A-H. These four Latin squares were stacked next to each other to receive a matrix of 8x32. Only entries A and B were kept, all others discarded. This resulted in a matrix with two entries (A and B) per column and eight (four times A and B) per line. Pea cultivars were then randomly assigned to columns and barley cultivars to lines and every combination that had either an A or a B was used. This totaled in 64 mixtures. See Haug *et al.* (2021) for theoretical background on the choice of an incomplete factorial design, Fig. A5 for the experimental design and Fig. A6 for an example of one trial layout (Fis 2019). After the removal of five pea genotypes (see 'plant material') the design was not balanced any more with certain barley genotypes being combined with only seven or six pea genotypes. Besides the mixed crops, pure stands of all used pea and barley genotypes, including GMs, were grown. Each experiment comprised two blocks (replications). In total, 712 experimental plots were sown, measured, and harvested. Yield data of 98.0 % of all plots could be used for analysis, while 2.0 % (14 plots) had to be removed due to sowing errors or non-maturity at harvest. Field emergence was counted on a plot level to document differences due to different seed sources. Counting took place three to five weeks after sowing and by counting twice 1m per plot. Genotypes with very low germination rates were excluded (see 'plant material'). Seed for the 2019 trial was farm-saved-seed from the 2018 trial, thus had uniform production conditions, and visual evaluation as well as a sample counting on 20% of the plots did not reveal significant differences in field emergence in 2019.

## 2.2. Experiment 2 (2020)

### 2.2.1. Plant material

Seventeen out of the twenty-two genotypes used in experiment 1 were used in experiment 2. A positive, as well as a negative selection was performed based on three selection methods for the choice of the 17 pea cultivars (see materials and methods section in Chapter 3 for a description of the three selection methods, p. 78ff; see Table 8, p. 79 for the list of genotypes used).

### 2.2.2. Sites, soils, and trial management

The experiment was carried out at two organically managed locations in Switzerland Fislisbach ('Fis') and Uster ('Ust'). For sowing densities in mixture and pure and row configurations see the sites, soils and trial management section in the materials and methods of Experiment 1. Mineral soil nitrogen (NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>) at sowing time ranged from 54.9 kg/ha (Ust) to 76.6 kg/ha (Fis) For detailed soil parameters, see Table A4, for climate conditions, see Fig. A3.

### 2.2.3. Experimental design

Seventeen pea-genotypes were combined with two barley genotypes to a full factorial trial. The barley cultivars were B5, cv. 'Atrika' and with the genotype mixture ('GM') B3, consisting of seven barley cultivars (see p. 32). In total 34 different mixtures per replication were sown. Per location one field trial consisted in three replications.

## 2.3. Measurements

Since pea was in the focus in this study, specific traits were measured in this species in addition to the yield recorded for the two species. Twelve traits were measured in pea pure stands: early vigor, early canopy height, early canopy height (2<sup>nd</sup> date), canopy height at grain filling (also measured in mixture), canopy height at maturity, onset of flowering, plant biomass, stipule length, stipule diameter, stipule area (derived from length and diameter), lodging at grain filling and lodging at maturity. Early vigor was assessed between BBCH 25 and 36 as a score from 1-9 with 9 given for highly vigorous plants. The score was a combined score of plant height (up to 4 points), earliness with having more than eight stretched internodes (1 point) and leaf area (up to 4 points). The four canopy-height measures were taken at end of leaf development (BBCH 19), stretching (BBCH34-39), grain filling (BBCH 74-79) and maturity (BBCH 85) with a leveling staff, measuring the height at which the canopy-horizon fully covered the staff. Onset of flowering was defined as the number of days after 1<sup>st</sup> of January when 50% of the plants of a plot had started flowering (BBCH 65). Plant biomass was assessed visually with a scoring from 1-9 with 9 being plots with the highest biomass at onset of flowering (BBCH 65) as well as stipule length, which was measured with a ruler from the two most distinct tips of the 2<sup>nd</sup> topmost fully developed stipule (Fig. 20) and stipule diameter measuring the broadest part of one stipule leaf. For the stipule area, an elliptical shape of the stipule was assumed and according to the ellipsis

formula the areas were calculated using length and diameter. For the stipule parameters, four representative plants were measured per plot. Stipule parameters were measured on the full set of genotypes in Fis 2018 and on a subset in the Fis 2019 experiment. Lodging was assessed in a 1-9 scale where 5 was a plot lodging in a 45° angle and 9 was a fully lodged plot. See Table A7 for an overview of the data structure and Fig. A8 for a correlation matrix of all measurements. After harvest, mixtures and pure stand plot-harvests were wind-cleaned and mixtures were separated in pea and barley fraction yields with a sieving-machine. Pea and barley fraction yields were measured, and moisture standardized (14%) after NIRS measurement of water content. The total yield was then computed as the sum of these two fraction yields. In some cases, a high amount of broken pea kernels was present in barley yield and a cutoff-value of >15% broken pea kernels were set for which the fraction yield data was not used, only total yield data.

### 3. Chapter 1 – A toolbox for breeding for mixed cropping: incomplete factorials, the producer/associate-concept and biological interaction functions

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#### 3.1. Introduction

The choice of the genotypes best suited to mixing within each species is not straight-forward, and a robust strategy for evaluating and identifying the right mixing partners from among a large number of candidates is essential to selecting the components of mixtures and improving the mixing ability of each species. Selection efficiency for mixed cropping yield under pure stand has been reported to be moderate or low, highlighting the value dedicated breeding efforts for mixed cropping (Annicchiarico *et al.*, 2019; de Oliveira Zimmermann, 1996; O’Leary and Smith, 2004). Well performing genotypes should display a high general mixing ability (GMA), i.e., lead to a high total mixture yield performance across several potential mixing partners, and a low variance in specific mixing ability (SMA), i.e., little or no specific interaction with individual mixing partners in that respect. In order to develop efficient breeding strategies for crop mixtures of two species, trial designs must be developed that allow precise estimation of GMA and SMA variance. Current trial designs apply a factorial setup, combining  $m$  genotypes of species one and  $n$  genotypes of species two. With increasing numbers of genotypes of both mixing partners, factorial designs quickly result in an unfeasibly high number of experimental plots. Therefore, often specific dimensions of both crop species are used: depending on the question to be addressed either (i) factorials of equal (or similar) dimensions of  $m$  and  $n$  with a small to medium number of genotypes (Annicchiarico and Piano, 1994; Hauggaard-Nielsen and Jensen, 2001) or (ii) factorials of different dimensions for  $m$  and  $n$  (Annicchiarico, 2003; Hoppe, 2016; Starke, 2018) are employed. The former allows GMA and SMA estimations of both crop species involved, the latter emphasizes one species over the other and is comparable to the topcross designs, used in hybrid breeding. With advances in mixed modeling statistical software (such as with the GNU R packages “lme4” or “SOMMER”), analyses of largely incomplete datasets are possible (Bates *et al.*, 2015, p. 4; Covarrubias-Pazarán, 2016; R Core Team, 2019). Incomplete factorial designs have been suggested to mitigate the limitations of (i) and (ii) by expanding the numbers of  $m$  and  $n$  while maintaining a feasible number of experimental plots. Previously, they have been applied to assess GMA and SMA effects in wheat (*T. aestivum* L.) cultivar mixtures (Forst *et al.*, 2019) and found recent application in genomic prediction in corn (*Z. mays* L.) hybrid breeding (Seye *et al.*, 2020). Another important area to advance breeding for mixed cropping focuses on exploiting



information that is contained in the fraction yields of mixed crops via the application of the producer (Pr) and associate (As) concept (Annicchiarico *et al.*, 2019; Goldringer *et al.*, 1994; Wright, 1985). In the mixed cropping context, the Pr effect, sometimes also referred to as direct effect, is the capacity of a genotype to withstand competition, to perform under competitive conditions in a mixture, while the As effect is its capacity to influence the yield of its companion crop or variety (Annicchiarico *et al.* 2019). As laid out by Wright (1985), Forst (2018) and Sampoux *et al.* (2020), the Pr and As effects of a given genotype sum up to its GMA effect. It has been applied to single row experiments in breeding nurseries (Goldringer *et al.*, 1994) or wheat cultivar mixtures (Forst, 2018), and to the mixed cropping context (Sampoux *et al.*, 2020; Wright, 1985). Separated yield data enables either uni- or bivariate (or multivariate) analysis, i.e. joint analysis of the two (or multiple) fraction yields. In clinical psychology as well as in livestock breeding, multivariate analysis procedures have successfully been applied in situations where traits were correlated, e.g. due to pleiotropy, and yielded higher precision for QTL detection than univariate approaches (Meier *et al.*, 2015; Sørensen *et al.*, 2003). In mixed cropping conditions, it can be assumed that errors of measurements are generally negatively correlated between the two crops, e.g., via compensation effects. Often in mixed cropping, one of the species is at a competitive disadvantage and genotypes of the species that is very non-competitive generally express a low GMA in mixtures (Annicchiarico and Piano, 1994; Corre-Hellou *et al.*, 2006a). However, the competitive ability of genotypes is obscured when only whole mixture yield is observed (Annicchiarico *et al.*, 2019). The assessment of fraction yields is not only important to identify these competitive abilities, but can also be applied to optimize a mixture towards a specific ratio, e.g. for feed nutrition or legume subsidies reasons, as is for example the case in Switzerland (BLW, 2019). Thus, shaping community performance and composition via traits can be of interest in the breeding process. Furthermore, high genetic correlations between certain traits and mixture yield would allow indirect selection based on most important key traits for Pr and As effects. The choice of an efficient trial design, the choice of an efficient analysis method and the assessment of yield proportions are interrelated topics. They provide the potential to be combined in an integrated approach to promote breeding for mixed cropping. While some published work focusses on the parallel genetic improvement of two species (Sampoux *et al.*, 2020), many publications rely on the improvement of one species at a time and do not take the potential of analyzing separated yield data into account. Thus, the objectives of this study were to (i) develop a model to estimate GMA and SMA variances of binary species mixtures, and to compare different experimental designs for their usefulness in estimating these parameters, (ii) subdivide GMA into Pr and As effects in order to categorize cultivars' influence on mixture yield and to compare the precision of a uni- versus a bivariate approach in estimating Pr, As and error (co)variances, and (iii) establish a concept to link plant traits to biological interactions between involved species in mixture.

## 3.2. Materials and methods

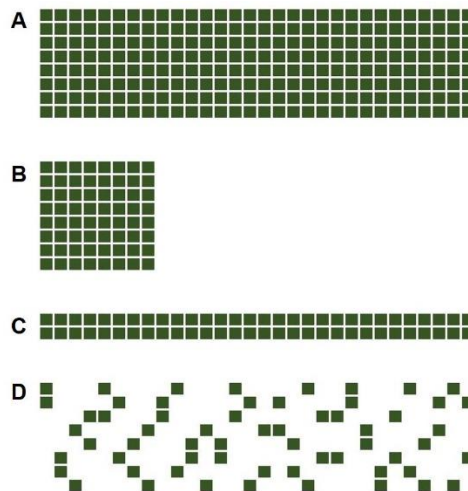
To exemplify the case of a mixed crop, a hypothetical binary mixture of a legume species (pea) and a non-legume species (barley) will be used in this study.

### 3.2.1. The GMA model of total mixture yield

Mixture yield can be expressed with the following model:

$$y_{ijk} = \mu + r_k + G_{p_i} + G_{b_j} + S_{ij} + E_{ijk} \quad (4)$$

with  $y_{ijk}$  the total mixture yield of the  $i$ -th pea cultivar mixed with the  $j$ -th barley cultivar in the  $k$ -th block,  $\mu$  the intercept of mixture yields,  $r_k$  the effect of the  $k$ -th block (replication),  $G_{p_i}$  and  $G_{b_j}$  the GMA effects the  $i$ -th pea cultivar and the  $j$ -th barley cultivar, respectively,  $S_{ij}$  the SMA effect, i.e. interaction, of the  $i$ -th pea cultivar with the  $j$ -th barley cultivar and  $E_{ijk}$  the error term. This model-framework was used to compare four different trial designs (A-D), comprising three full ("f") factorials, with all possible pairwise combinations present, and an incomplete ("i") factorial with only a subset of all possible pairwise combinations present (Fig. 1). In the following,  $m$  is the number of barley cultivars and  $n$  the number of pea cultivars, used in the design. Design A, using 240 plots per replicate ( $m=8$  and  $n=30$ ), is the most resource-expensive design.



**Fig. 4.** The four experimental designs used in this study, comprising three full (f) factorials with all possible pairwise combinations present and an incomplete (i) factorial with only a subset of all possible pairwise combinations present. The designs included (A) an 8 barleys x 30 peas full factorial (8x30f), (B) an 8 barleys x 8 peas full factorial (8x8f), (C) a 2 barleys x 30 peas full factorial (2x30f) and (D) an 8 barleys times 30 peas incomplete factorial (8x30i). The last three designs consume roughly the same amount of experimental resources with 64, 60 and 60 experimental plots, respectively.

Designs B, C and D were using only approximately 25% of the resources of A with respectively 64 ( $m=8$ ,  $n=8$ ), 60 ( $m=8$ ,  $n=30$ ) and 60 ( $m=2$ ,  $n=30$ ) plots per replicate, while sharing commonalities with design A. Designs B and C are both also full factorials (with equal and unequal dimensions of  $m$  and  $n$ ) and design D shares the same size of  $m$  and  $n$  with design A

while being an incomplete factorial. Design C (2x30f) full factorial has similarities with a topcross design, used in early stages of a hybrid breeding program. Design D (8x30i) was constructed using four independently randomized 8x8 Latin squares of which only two "entries" were used for the mixtures. This ensured that a) every barley was combined with eight pea cultivars, and every pea was combined with two different barley cultivars and b) confounding, i.e. two or more pea cultivars sharing the same two barley cultivars was minimized. For the comparison of the four designs, datasets with total mixture yield-data ("total yield setting") were simulated with the following models for a "SMA present" and a "SMA absent" simulation, respectively. For the SMA absent simulation,  $S_{ij}$  was set to zero in the model in formula (4). Simulations were performed according to the following procedure, using parameter settings in the same order of magnitude as empirical values from preliminary trials (Haug *et al.*, in preparation) to produce data that is as close to empirical data as possible. The intercept of mixture yield was set to 38.8 dt/ha. For each of the following parameters, the corresponding effects were drawn from their respective probability distributions: block effects  $r_k$  were drawn from a normal distribution with a mean of 0 and a variance of 2, i.e.,  $N(0, 2)$ . Pea GMA effects  $G_{pi}$  were drawn from  $N(3, 0)$ , barley GMA effects  $G_{bj}$  were drawn from  $N(0, 5)$  and SMA effects  $S_{ij}$  were drawn from  $N(0, 5)$  for the SMA present simulation and from  $N(0, 0)$  for the SMA absent simulation, i.e., the effect size was set to zero. Errors  $e_{ijk}$  were drawn from  $N(0, 5)$ . For each simulation run, effects were drawn anew. "True" values (i.e., values used to simulate data) of each effect were saved after each simulation run for later comparison with the estimated parameter values, e.g. the true GMA effects of the pea cultivars, were stored for later comparison with GMA effects estimated by the best linear unbiased predictors (BLUPs) received by the mixed model to analyze the simulated data. Design D normally would have 64 pairwise combinations. The realistic case of missing/unusable genotypes was assumed, by excluding two pea lines in our case, thus resulting in 60 pea-barley combinations. For each of the four trial designs  $n=1000$  data sets were simulated, for the SMA present, as well as the SMA absent simulation, i.e.,  $8 \times 1000$  simulated data-sets in total. Each dataset comprised two replicates (blocks). All simulations and subsequent analyses were done using GNU R (R Core Team, 2019). The R-code used for simulation and analysis is publicly available (Haug, 2020a).

### 3.2.2. The Pr/As model of fraction yield of each species

Effects on separated yield data, i.e., pea and barley fraction yields, can be described by a model, containing producer (Pr) and associate (As) effects:

$$y_{p_{ijk}} = \mu_p + r_{p_k} + P_{p_i} + A_{b_j} + E_{p_{ijk}} \quad (5)$$

$$y_{b_{ijk}} = \mu_b + r_{b_k} + P_{b_j} + A_{p_i} + E_{b_{ijk}} \quad (6)$$

with  $y_{p_{ijk}}$  the fraction yield of pea from the combination of the  $i$ -th pea cultivar with the  $j$ -th barley cultivar in the  $k$ -th block,  $\mu_p$  the intercept of pea fraction yields,  $r_{p_k}$  the effects of the  $k$ -th block on pea fraction yield,  $P_{p_i}$  the Pr effects and  $A_{b_j}$  the As effects of the  $i$ -th pea and the  $j$ -th barley cultivar, respectively, and  $E_{p_{ijk}}$  the error for the fraction yield of the  $i$ -th pea with the  $j$ -th barley in the  $k$ -th block. Each dataset comprised two replicates (blocks). Parameters apply in analogy for barley fraction yields in formula (6). Interactions between Pr and As effects are ignored. Since total mixture yield  $y_{ijk}$  decomposes into  $(y_{p_{ijk}} + y_{b_{ijk}})$ , also the other parameters can be decomposed:  $\mu$  into  $(\mu_p + \mu_b)$ ,  $r_k$  into  $(r_{p_k} + r_{b_k})$ ,  $G_p$  into  $(P_p + A_p)$ ,  $G_b$  into  $(P_b + A_b)$ , and  $E_{ijk}$  into  $(E_{p_{ijk}} + E_{b_{ijk}})$ . Hence, formula (4) (without  $S_{ij}$ ) can be rewritten as in formula (7).

$$y_{p_{ijk}} + y_{b_{ijk}} = \mu_p + \mu_b + r_{p_k} + r_{b_k} + P_{p_i} + A_{p_i} + P_{b_j} + A_{b_j} + E_{p_{ijk}} + E_{b_{ijk}} \quad (7)$$

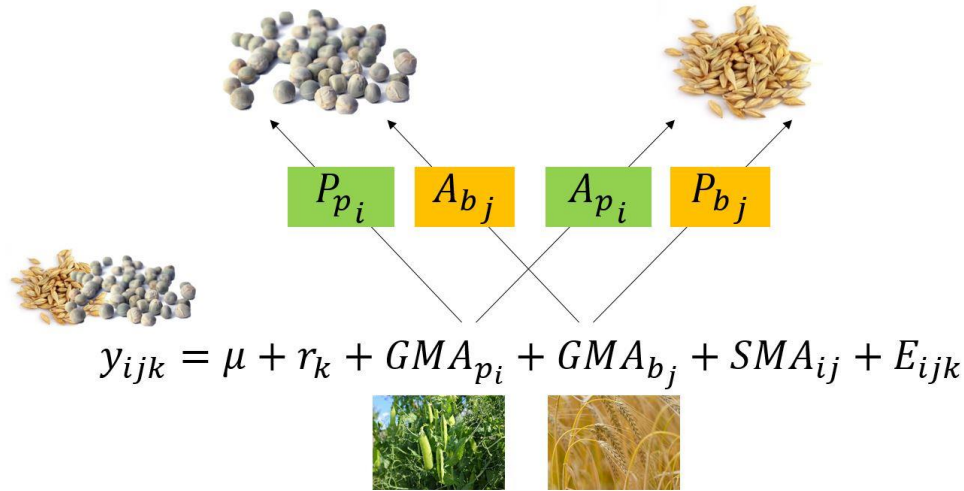
$$\begin{bmatrix} P_{p_i} \\ A_{p_i} \end{bmatrix} \sim N_2 \left( \begin{bmatrix} 0 \\ 0 \end{bmatrix}, \begin{bmatrix} 6.1 & -4.1 \\ -4.1 & 7.5 \end{bmatrix} \right) \quad (8)$$

$$\begin{bmatrix} P_{b_j} \\ A_{b_j} \end{bmatrix} \sim N_2 \left( \begin{bmatrix} 0 \\ 0 \end{bmatrix}, \begin{bmatrix} 6.1 & -2.7 \\ -2.7 & 1.5 \end{bmatrix} \right) \quad (9)$$

$$\begin{bmatrix} E_{p_{ijk}} \\ E_{b_{ijk}} \end{bmatrix} \sim N_2 \left( \begin{bmatrix} 0 \\ 0 \end{bmatrix}, \begin{bmatrix} 6.1 & 3.6 \\ 3.6 & 8.4 \end{bmatrix} \right) \quad (10)$$

Decomposition of GMA into Pr and As effects is illustrated in Fig. 5. Separated yield data ("fraction yield setting") were simulated to compare the precision of a univariate versus a bivariate analysis approach for design D (Fig. 4). As in the total yield setting, two blocks per data set were simulated.

In contrast to the total yield setting, for each “plot” the separated yields of pea and barley were simulated with a mean pea yield of 18.3 dt/ha and a mean barley yield of 19.1 dt/ha, according to previously mentioned preliminary experimental data.



**Fig. 5.** Decomposition of the general mixing ability of pea ( $G_{p_i}$ ) in its producer ( $P_{p_i}$ ) and associate ( $A_{p_i}$ ) effects. Parameters apply in analogy for barley.

The following settings were used: block effects  $r_{p_k}$  and  $r_{b_k}$  were both drawn from  $N(0, 2)$  as in the total yield setting. Pea Pr effects  $P_{p_i}$  were drawn from  $N(0, 6.1)$  and As effects  $A_{b_j}$  from  $N(0, 7.5)$ . Barley Pr effects  $P_{b_j}$  were drawn from  $N(0, 6.1)$  and As effects  $A_{p_i}$  from  $N(0, 1.5)$ , errors  $E_{p_{ijk}}$  and  $E_{b_{ijk}}$  were drawn for pea yield from  $N(0, 8.4)$  and for barley yield from  $N(0, 6.1)$ . Correlations of pea Pr effects with pea As effects were set to -0.61, and correlation of barley Pr with barley As effects were set to -0.81. Within-plot error-correlations, i.e., the correlation of errors of barley yields with the errors of the corresponding pea yields in the same plot, were set to 0, -0.5 and -0.9, respectively, to create three different error-correlation scenarios. Within-plot error-correlations and Pr/As correlations were translated into co-variances. The variance-covariance matrices of Pr/As effects of pea, barley and the errors are shown in formulas (8), (9) and (10). Pr and As effects as well as errors were drawn from a distribution that follows the law of a multivariate normal distribution, using the function “mvrnorm” from the R-package “MASS” (Venables and Ripley, 2002), and the covariances shown in formulas (8) to (10). In total, three data sets for each of the three different error correlation settings with 1000 simulations were created.

Simulated data from the fraction yield setting was analyzed using (i) a univariate approach with models equal to those used to simulate the data (formulas (5), (6)) and (ii) a bivariate approach (formula (7)) in which the two dependent variables were analyzed jointly (Covarrubias-Pazaran, 2018). In addition to the parameters estimated by the univariate approach, the bivariate approach also estimates the before mentioned covariances. Both approaches were done as mixed models where block-effects were considered as fixed and all other effects as random, assuming independent and identically distributed random variables. The uni- and the bivariate

analyses were done with the "mmer" function of the R-package "SOMMER" (Covarrubias-Pazaran, 2016, Covarrubias-Pazaran, 2018). Estimates of the model parameters, e.g., estimated GMA or Pr variances of pea, and BLUPs for the genetic effects, e.g., BLUPs of GMA effects, were saved for later analysis for each of the 1000 datasets per setting. Depending on the analysis approach, each dataset yielded a different set of BLUPs for the pea and barley producer and associate effects. For n=1000 analyses, Pearson correlations between the two sets of BLUPs and the true value were computed, Fisher-z-transformed, averaged for each coefficient and transformed back. T-tests between the mean correlation of the univariate and the bivariate approach within each parameter were conducted to compare the approaches for their accuracy to estimate the true effect values. The R-code used for simulation and analysis is publicly available (Haug, 2020b).

### 3.2.3. Trait versus GMA/Pr/As analyses for the characterization of biological interaction functions (BIFs)

Beyond the purely statistical treatment of the data described above, the relationships between a fictive explanatory trait and the GMA/Pr/As variables were investigated. This explanatory trait was set into relation with the GMA, Pr and As effects of equally fictive genotypes. Nine possible scenarios of trait-GMA, trait-Pr effect and trait As-effect relationships were investigated, allowing the categorization of traits according to their biological interaction function in a mixed-crop plant community. Exemplary scatterplots with n=100 simulated genotypes and positive, null and negative relationships between trait and GMA, trait and Pr and trait and As effects were created to suggest a simple visual analysis of these relationships. Potential symbiotic trait functions were associated to the corresponding functions relationships.

## 3.3. Results

### 3.3.1. Incomplete design yields comparable estimates to full factorial designs

Four experimental designs were compared for their ability to estimate GMA and SMA variances as well as estimating genotypic effects (BLUPs) correctly in two different simulations, a "SMA-present" and a "SMA-absent" simulation. Over both scenarios, the precision of the estimates increased with experimental resource input (Table 5). With design A (8x30f), utilizing 240 experimental plots per replicate, the narrowest CIs among the four designs were received, narrower than the ones of designs B (8x8f), C (2x8f) and D (8x30i), which were using only 64, 60 and 60 experimental plots per replicate, respectively (Table 5). Among the latter, only minor differences in CIs were observed (except lower reliability on GMA variance of barley in design C and on GMA variance of pea in design B in the SMA-absent simulation). Besides GMA variance of barley of design C in the SMA-present simulation and the GMA variance of pea of design B in the SMA-absent simulation, certain parameters were estimated similarly well with designs B, C and D as with the benchmark design A. Barley GMA variance of designs B and D showed similar

CIs compared to design A for both the SMA-present and the SMA-absent simulation, whereas design C, which only uses two instead of eight barley cultivars, estimated barley GMA variance less precisely (CI of  $\pm 0.43$  in both scenarios). In addition, pea GMA variance of designs C and D of the SMA-absent simulation were similarly precisely estimated compared with design A, whereas design B estimated this parameter with lower precision (CI of  $\pm 0.11$ ). Besides the variation of estimates, a check of the correct estimation of the size of the parameter itself revealed that for the SMA-present simulation (Table 5), all four experimental designs accurately estimated GMA, SMA and error variances, with all confidence intervals (CIs) of means overlapping the true values, except for the SMA variance of design C. For the SMA-absent simulation, pea and barley GMA variances were mostly accurately estimated in the four designs with significant but small underestimations of pea GMA variance by designs C and D. SMA variances in this simulation were significantly overestimated and error variances were significantly underestimated for all four designs. Compared with the mean SMA variance of the benchmark design A of 0.14, in this simulation designs B, C and D showed significantly higher SMA variances with 0.27, 0.35 and 0.36, respectively. Similarly, the mean error variance of design A was with 4.88 significantly higher (and thus closer to the truth of 5.0) than the error variances of designs B, C and D, with means between 4.76 and 4.80.

**Table 5.** Results on general and specific mixing ability (GMA and SMA) from simulated data for four different trial designs (A-D). The four designs employ different amounts of resources in terms of experimental plots per replicate, with design A being the benchmark. Numbers after  $\pm$  denote the 95% confidence intervals for the mean of 1000 variance estimates. Different letters following correlation values between best linear unbiased predictors (BLUPs) and true effects indicate significant differences (Tukey-HSD;  $p < 0.05$ ).

Simulation	Trial design <sup>1</sup>	Plots per replicate	Pea GMA		Barley GMA		SMA		Error	
			Means of variance estimates (truth: 3.0) <sup>2</sup>	Means of correlations BLUPs vs. true effects <sup>3</sup>	Means of variance estimates (truth: 5.0)	Means of correlations BLUPs vs. true effects	Means of variance estimates (truth: 5.0/0.0)	Means of correlations BLUPs vs. true effects	Means of variance estimates (truth: 5.0)	
SMA-present simulation	A (8 x 30 f)	240	2.97 $\pm$ 0.06	0.88 <sup>c</sup>	4.98 $\pm$ 0.17	0.98 <sup>c</sup>	5.00 $\pm$ 0.05	0.77 <sup>d</sup>	5.02 $\pm$ 0.03	
	B (8 x 8 f)	64	2.90 $\pm$ 0.13	0.89 <sup>d</sup>	4.98 $\pm$ 0.19	0.93 <sup>b</sup>	5.03 $\pm$ 0.10	0.74 <sup>c</sup>	5.02 $\pm$ 0.06	
	C (2 x 30 f)	60	3.06 $\pm$ 0.12	0.68 <sup>b</sup>	4.64 $\pm$ 0.43	- <sup>4</sup>	4.85 $\pm$ 0.12	0.70 <sup>b</sup>	5.03 $\pm$ 0.00	
	D (8 x 30 i)	60	3.01 $\pm$ 0.13	0.65 <sup>a</sup>	5.13 $\pm$ 0.21	0.91 <sup>a</sup>	4.95 $\pm$ 0.13	0.67 <sup>a</sup>	5.02 $\pm$ 0.06	
SMA-absent simulation	A (8 x 30 f)	240	2.98 $\pm$ 0.05	0.95 <sup>c</sup>	4.94 $\pm$ 0.17	0.99 <sup>c</sup>	0.14 $\pm$ 0.01	- <sup>5</sup>	4.88 $\pm$ 0.02	
	B (8 x 8 f)	64	2.90 $\pm$ 0.11	0.96 <sup>d</sup>	4.94 $\pm$ 0.18	0.98 <sup>b</sup>	0.27 $\pm$ 0.02	-	4.76 $\pm$ 0.04	
	C (2 x 30 f)	60	2.89 $\pm$ 0.07	0.84 <sup>b</sup>	4.64 $\pm$ 0.43	- <sup>4</sup>	0.35 $\pm$ 0.03	-	4.76 $\pm$ 0.05	
	D (8 x 30 i)	60	2.84 $\pm$ 0.07	0.83 <sup>a</sup>	4.89 $\pm$ 0.18	0.96 <sup>a</sup>	0.36 $\pm$ 0.03	-	4.80 $\pm$ 0.05	

<sup>1</sup>the different designs as described in Figure 1 <sup>2</sup>means of 1000 estimates per design and 95% confidence intervals <sup>3</sup>means of rho-z-rho transformed Pearson correlations, see materials & methods, letters a-d indicate significant differences with  $p < 0.001$  <sup>4</sup>correlation of only two data-points is by definition one <sup>5</sup>correlations were not computed in scenario two, for the true SMA-effects all being 0 by definition

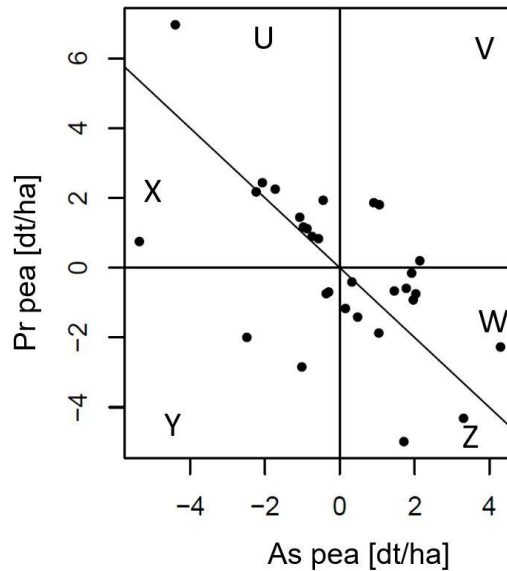


When comparing the four designs for their correlation of BLUPs with the truth value, (Table 5) the correlation coefficients of benchmark design A were among the highest across all estimated BLUPs. However, correlations of BLUPs of pea GMA effects with the true effects of design B were similar compared with the benchmark design A for both simulations. For the correlations of BLUPs for pea GMA with their true values of designs C and D showed significantly lower correlation coefficients compared with design A and B for both SMA simulation models. However, this difference was less apparent in the SMA-absent simulation with correlation coefficients of 0.84 and 0.83 of designs C and D compared with 0.95 and 0.96 of designs A and B, respectively. All correlation coefficients differed significantly from each other ( $p < 0.001$ ). For barley GMA, design B and D showed with 0.93 and 0.91 high mean correlation coefficients that were similar to the mean of design A (0.98). Correlation coefficients for SMA effects were in the range of the correlation coefficients for pea GMA effects and barley GMA effects, with values between 0.67 (design D) and 0.77 (design A).

### 3.3.2. The Pr/As concept allows to characterize cultivars' contribution to mixture yield

In Fig. 6, thirty simulated cultivars with their Pr and As effects are shown. Pr effects range from -5.0 to +7.0 and As effects range from -5.4 to +4.3. As effect has to be read as the effect of the species (e.g., pea) on the yield (or any other trait) of its companion species (e.g., barley). Since the Pr and As effects of a cultivar sum up to its GMA, cultivars that lie on the line with slope -1 and intercept 0 have a GMA of zero, those above this line have a positive and below a negative GMA. The cultivars thus can be grouped into six sectors, U, V, W, X, Y and Z, with U-W having positive GMA due to a high Pr effect that offsets a negative As effect (sector U), both a positive Pr and As effect (sector V) and a positive As effect that makes up for a negative Pr effect (sector W). On the other hand, cultivars below the identity line have a negative GMA with a positive Pr effect that does not compensate for a negative As effect (sector X), both negative Pr and As effects (sector Y) and a negative Pr effect which is not offset by their positive As effect (sector Z). These six sectors allow to characterize and differentiate the mixing ability of the pea cultivars.






















Comparing the uni- and bivariate approach using the data from the fraction yield setting, correlation of errors of 0, -0.5 and -0.9 resulted in remarkably similar parameter estimates (Table A1, p. 89). The "error correlation of -0.5 scenario" was used to analyze separated fraction yield data of both models in more detail. Both analysis approaches, uni- and bi-variate, produced unbiased results of parameter estimates, i.e., all 95% confidence intervals of the estimates contained the true values for both approaches (see Table A2, p. 90). Estimates did not differ significantly between the univariate and the bivariate analysis approach. CIs, used as a measure for precision, differed only by 0.01 or not at all. However, only the bivariate model allows to estimate the correlation between Pr and As effects.



**Fig. 6.** Best linear unbiased predictors of producer (Pr) and associate (As) effects of 30 pea cultivars of a bivariate analysis of simulated data in which an 8 barleys x 30 peas incomplete factorial design was used (Design C). Pr and As effects represent the yield effects in dt/ha of cultivars of a focal species (here pea) on partial yields of itself (Pr) or on the associated species (As; here barley; read "As effect of pea on barley yields"). Data taken from a randomly chosen simulated data set of the Pr/As data. The sum of the Pr and As effects of a cultivar equals its GMA effect, thus, the line with slope -1 and intercept 0 separates genotypes with positive (above) and negative (below) GMA. Genotypes can have a positive GMA by either a high Pr effect that offsets for a negative As effect (sector U), both positive Pr and As effects (sector V) or a high As effect that offsets for a negative Pr effect (sector W; consequently opposite for sectors X, Y and Z).

### 3.3.3. Pr/As-trait relationships uncover biological interaction functions (BIFs) of traits

The GMA, Pr and As effects on total or fraction yield do not reveal the underlying biological processes or traits that influence the mixing ability. Yet, the examination of relationships between a fictive explanatory trait and Pr/As effects on fraction yield fills this lack by defining nine potential biological interaction functions (BIFs) of a given trait that underlie the GMA-trait pattern (Fig. 7). This GMA-trait relationship can be positive (+), absent (0) or negative (-). However, the GMA-trait relationship is subdivided in its underpinned three possible Pr-trait/As-trait relationships. These can then be interpreted in terms of BIFs: commensalism (Pr+/As0, Pr0/As+, i.e. trait will profit only one species), mutualism (Pr+/As+, i.e., trait promotes both species), antagonism (Pr+/As-, Pr-/As+, i.e., trait promotes one species but hampers second species), neutralism (Pr0/As0, i.e. trait does not affect any of the two species), amensalism (Pr0/As-, Pr-/As0, i.e. trait is hampering only one species) and competition (Pr-/As-, i.e. trait is hampering both species). This more detailed correlations will allow to identify key traits that are important for good mixing ability for a crop and can contribute to indirect selection.

$GMA_{pea}$	$Pr_{pea}$	$As_{pea}$	Biological interaction-function (BIF) of pea trait	Pattern
			Commensalism	+/0
			Commensalism	0/+
			Mutualism	+/+
			Antagonism	+/-
			Antagonism	-/+
			Neutralism	0/0
			Amensalism	0/-
			Amensalism	-/0
			Competition	-/-

**Fig. 7.** Schematic representation of pea genotypes with three potential relationships (positively correlated, uncorrelated, negatively correlated) of their GMA with a fictive explanatory trait and three potential underlying Pr- and As-trait relationships. Values of the explanatory trait lie on the x-axis, GMA, Pr and As values on the y-axis. Pr/As-trait relationships reveal different biologic interaction functions (BIFs). The pattern describes a neutral (0), positive (+) or negative (-) influence on the species on which the trait was measured (left of the slash) or the species associated to this species (right of the slash).

### 3.4. Discussion

The overall goal of this study was to develop a novel framework for breeding for mixed cropping by (i) formulating models for mixed cropping, suggesting experimental designs and analysis methods for mixed cropping experiments, (ii) proposing extensions to the use of the Pr/As concept, and (iii) linking the latter to traits in order to uncover the biological interaction function (BIF) of traits.

#### 3.4.1. Incomplete designs to increase selection intensities

With all four designs, GMA, SMA and error variances were overall correctly estimated, i.e. with little or no bias. As expected, the low-resource designs B, C and D showed a slightly lower precision (i.e. higher CIs). The comparison of the three low-resource designs, incomplete factorial design (D) versus the two full factorial designs B and C, revealed similar estimations of GMA, SMA and error variances. Design C (with 2 barley genotypes) does not allow a meaningful estimation of GMA variance. Thus, for an estimation of both species' GMA variance and SMA

variance, designs B or D are preferred, which is in line with the suggestion of Annicchiarico *et al.* (2019). Seye *et al.* (2020) used an incomplete factorial design for hybrid testing that was created by crossing one inbred line of one pool with one inbred line from the opposite other pool and compared to a classical topcross design where all inbred lines of pool 1 were crossed with the same line of pool 2. In the incomplete design, estimates for general combining ability (GCA) for both parents of a hybrid cannot be disentangled and are identical. Nonetheless, they emphasize, that if one only considers selection among the tested lines of the training set, the incomplete factorial (similar to design D of this study) always outperformed a topcross design (similar to design C) in terms of genetic gain, since twice the number of genotypes (similar to barley in our case) could be tested and consequently selection intensity could be twice as high.

Correlations of true with estimated pea GMA values are lower in the incomplete design D compared with design B. In the absence of SMA however, correlations come close to those of design B and even high-resource design A. This suggests that incomplete factorials can combine the advantages and applications of both designs with equal or similar dimensions of  $m$  and  $n$  and of designs with unequal dimensions of  $m$  and  $n$ , the latter being like topcross designs in hybrid breeding. At similar resource requirements, incomplete factorials allow more genotypes to be tested without a substantial loss of GMA precision and accuracy in cases where SMA is negligible. This will allow to increase selection intensity like the example shown in maize hybrid breeding (Seye *et al.* 2020). Moreover, testing larger sets of genotypes allow to exploit larger genetic variance of a given species. Since selection gain depends on both intensity and genetic variance, incomplete designs have a great potential to increase selection gain when breeding for mixed cropping.

### 3.4.2. Incomplete designs for early and later stages of breeding for mixed cropping

Besides having been suggested for calibration of genomic prediction models for hybrid breeding (Seye *et al.*, 2020), incomplete designs have been used to estimate GMA and SMA effects in wheat cultivar mixtures (Forst *et al.*, 2019). The findings of Forst *et al.* (2019) could be applied to hybrid breeding as well as to breeding for mixed cropping: in the early development of a hybrid selection scheme for a crop, a broad range of genotypes could be tested in an incomplete diallel, like the one in Forst *et al.* (2019) that identified suitable material to form "pools" in cultivar mixtures. In mixed cropping, in early stages of breeding, where the size of the GMA variances of the two species are yet unknown and both species are of equal interest, an incomplete factorial with equal sizes of  $m$  and  $n$  would be advisable to subsequently design a breeding scheme based on the results. In later stages of both hybrid breeding (heterotic pools have been formed) and breeding for mixed cropping (focal species has been chosen), an incomplete factorial, e.g., in the form of design D, could be applied to both pools (hybrid breeding) or the focal species (mixed cropping). Only little literature has been published on actual experiments for breeding for mixed cropping. Some authors focus on the stepwise approach, first conducting a topcross design

(similar to design C) to identify most promising genotypes for mixtures, followed by a full factorial to identify best combinations (similar to design B) for the development of two components of a mixture, such as species mixtures of maize (Hoppe, 2016) or common bean (Starke, 2018). The results presented in this study suggest such stepwise experiments could have been combined to one by the application of an incomplete design, thus speeding up the selection process. Incomplete designs can be applied to similar problems where factorial experimental designs are used, notably hybrid breeding, animal breeding.

### 3.4.3. Pr/As concept to select genotypes according to their species-specific mixing ability

The Pr/As concept allows to cluster genotypes into groups of particular "mixing-behaviors" within positive or negative GMA. Therefore, depending on the desired proportion of fraction yield by farmers, in our example a larger ratio of pea to barley, pea genotypes can be selected from either sectors U or V of Fig. 6, while pea genotypes of sector W would support a higher proportion of barley. The Pr/As concept also allows to select for GMA maximization via a regression of the Pr effects on the As effects, i.e. fit a regression to the Fig. 6 dataset. For instance, a regression with a slope strictly steeper than -1 (e.g., -1.5) indicates total yield can be increased by more competitive cultivars of the focal species, thus, GMA is maximized via the selection towards higher Pr or lower As effects. The Pr/As concept can be seen as the genetic correspondence to the replacement series as, for example, described by Wendling *et al.* (2017). They compared four different crop species in pairwise combinations for their biomass yield under mixed cropping. Both the Pr/As concept and replacement series describe levels of competitiveness between two species under varying competitive conditions within the mixture, conveyed by different sowing ratios in the replacement series, and by genetic differences in competitiveness in the Pr/As concept. The low and high sowing ratios of a species in the replacement series would then correspond to low (positive As effects) or highly competitive (negative As effects) genotypes, respectively. Wendling *et al.* (2017) observed linear relationships between mixed crop species only in two out of twelve replacement series. For all other scenarios, local maxima with transgressive overyielding were identified, i.e., mixture biomass yield exceeded the pure-stand biomass yield of each species. Due to the resemblance of the two concepts, it is quite possible, that similar local maxima for mixture yield occur in a Pr/As context. In this case, instead of a linear regression, bi-, polynomial, local or non-parametric regressions could be applied, in order to find a target interval for As values to maximize mixture yield. Pr and As effects are correctly estimated without being biased by different levels of correlated errors. A bivariate model is provided, able to take such a correlation into account. This model is an original analysis approach and the canonical way to treat paired variables that are presenting obvious inter-dependencies (yield of two crops cultivated on the same plot). However, this bivariate approach, with our design chosen to ensure balance and avoid confounding as much as possible, did not yield an improvement in terms of precision of estimates compared with the univariate approach. This is against our expectation and suggesting literature (Meier *et al.*, 2015; Sørensen

*et al.*, 2003) but the focus of this study was on parameter estimation, whereas strengths of bivariate approaches might rather lie in other applications, such as hypothesis testing and outcome prediction. Even though the precision of estimates could not be improved with it, the multivariate approach can be used to estimate genetic correlations between traits (Meyer, 1991), which is fundamental for the use of indirect selection methods in mixed crops as suggested by Annicchiarico *et al.* (2019).

#### 3.4.4. Pr/As-trait relationships to shape species mixtures

The Pr/As concept can be seen as an extension of the concept of competitive effect and response (Goldberg and Fleetwood, 1987). There, a relationship, or effect of trait A (e.g., early vigor), measured in species one (competitive effect) on a different trait B (e.g. yield), measured in species two (competitive response) is assumed. In the Pr/As context however, trait A in species one (e.g., early vigor) can have an effect on a trait B that is common in both species (e.g. yield of species one and two, i.e. Pr/As effects), as visualized in Fig. 7. By combining the Pr/As concept with trait measurements, the BIF of a trait can be determined. This bears the potential for further systematic investigation and categorization of trait functions in mixed cropping and community ecology, where it might serve to discover, which trait categories prevail in successful plant or other organismic communities and shape the functioning – or non-functioning – coexistence of these. The identification of BIFs is therefore important for breeding for crop mixture, which is not possible, if only the trait-GMA relationship (i.e., total yield) is being looked at. Nevertheless, analyzing correlations between GMA and traits can still be of interest to identify key traits that influence total mixture performance like in forage crops but not the performance of individual species.

## 4. Chapter 2 – Filling the niche with diversity

This chapter is currently being prepared to be submitted as an original research article to a peer-reviewed journal with a presumed submission in spring 2022.

### 4.1. Introduction

The determination of the ratio of variance that is caused by GMA and SMA in mixture yields as well as their absolute values of variance are essential to determine a breeding strategy for mixed cropping (Annicchiarico *et al.*, 2019; Hoppe, 2016). A predominance of GMA over SMA allows all genotypes of both species to be combined without deviating effects to be expected. This largely simplifies breeding efforts and subsequent seed marketing and handling by farmers. In breeding, after a test for GMA, specific combinations of genotypes do not have to be verified any more (test for SMA) and thus reduces experimental effort. In seed marketing this creates flexibility for retailers as they can combine seeds of both species by their preference. Finally, farmers can combine genotypes of both species without having to fear unexpected behavior by the choice they've made. The absolute size GMA determines the heritability of total mixture yields and thus the genetic leverage breeders have to influence mixture yield by means of breeding and its heritability. A low heritability for total yield is expected, given the known compensatory effects between species in mixed crops cause any positive or negative effect of a genotype to be buffered by the companion species and thus masking the genotype's effect on total yield. Nonetheless, the fact that niche complementarity effects in mixed crops exist, especially for light use (Barillot *et al.*, 2014; Bedoussac and Justes, 2010a), indicate that unused potential lies within breeding genotypes that use the available niche-space more efficiently than others. While not observed in corn (*Z. mays*) - climbing bean (*Ph. vulgaris*) mixed crops (Hoppe, 2016; Starke, 2018), SMA, however, was present in a faba bean-wheat experiment (Siebrecht-Schöll, 2019). The only study we found, combining several genotypes of pea with more than one barley did not report estimates for pea genotype x barley genotype interactions (Hauggaard-Nielsen and Jensen, 2001) and values for GMA and SMA of pea and barley are thus unknown. Breeding efforts on most crops today focus on pure-stand yield performance. Maybe this situation will change with increasing popularity of mixed cropping, but if so, this will only happen progressively. It is thus unlikely that breeding efforts of complete breeding programs will focus mainly on mixed cropping. It is more likely that, in a first step, breeding will focus on 'dual-use' genotypes that perform well in both ways of usage and that indirect selection by pure-stand yields will be used or that breeding strategies will split testing environments for pure- and mixed-stand cultivation at relatively late stages of the breeding scheme, since pure-stand cultivation will still be the main target environment for the nearer future. Therefore, the question to what extent pure-stand yield performance is correlated with mixed-stand yield performance is one of imminent importance. Based on the extent of the predictive capacity of pure stand yields, either no dedicated breeding programs (high correlation) or completely independent dedicated breeding programs have to

be set up (low correlation) or something in between (medium correlation). Annicchiarico *et al.* (2019) report in a small case study of (genetic) correlations between pure stand and mixed stands values that lay between 0.52 and 0.83, however, pea is not included in this overview. A literature review did not reveal any study reporting correlations between pea pure stand and mixed stand yield. While this is based on resource complementarity and adaptability conveyed by the functional diversity of mixed crops (Litrice and Violle, 2015), the stability of mixture components, i.e. of fraction yields has not yet been addressed in literature. The stability of fraction yields and consequently of the ratio of the two or more components, however, is of importance, as the ratio of marketable legume or cereal grains in the harvested lot is of economic significance for the farmer (Dierauer *et al.*, 2017; Viguier *et al.*, 2018). GMA and SMA both require information on total mixture yield. If separated yield data of both species is available ('fraction yields') the relative effects of the genotypes of each species on these fraction yields and, consequently, on the yield ratios can be investigated. The producer ('Pr') effect of a genotype is defined as the effect a genotype has on its own fraction yield in a mixture, relative to the overall mean of the fraction yields of its species. The associate ('As') effect of a genotype is defined as the effect of this genotype on the fraction yield of its companion species, relative to the overall mean of the fraction yields of the companion species. Pr effects have also been called 'direct' effects in literature (Annicchiarico *et al.*, 2019; Gallais, 1976), while As effects have correspondence to indirect interspecific genetic effects (IIGEs; Bailey *et al.*, 2014). Pr and As effects can be of help to understand genotypes' impact on mixture ratio as well as the impact of traits on the biological interactions taking place within a mixture (Haug *et al.*, 2021). While extensive research for using heterogeneous material within mixed crops of herbaceous legume species (for review Annicchiarico *et al.*, 2019) and for intraspecific diversity, e.g. cultivar mixtures, composite-cross populations and others, exist (Clay and Allard, 1969; Döring *et al.*, 2015; Knott and Mundt, 1990) little research has been done on the use of heterogeneous material within annual mixed crops. A literature search including the keywords 'multiline', 'multi cultivar', 'multi genotype', 'mixed cropping' and 'intercropping' did reveal two articles dealing with such an experimental setup. The first study was investigating six pea genotypes and their six possible binary genotype mixtures (GMs) together with barley (S. Darras *et al.*, 2015). The findings of this study were not consistent over experimental environments, however, and only yield advantage over pure stands was considered and GMs were not compared to homogeneous genotypes for their stability. The second study (Carlsson *et al.*, 2017), used two different pea GMs, consisting of two genotypes each, the first one combining a semi-leafless type (cv. Partner) with a leafy type (cv. Dukat), the second, using two leafless types (cv. Dove and cv. Balltrap). The standard error (as a measure of yield instability) did not seem to be smaller for GMs as compared to single cultivars for pure stand yields, possibly due to strong lodging of one of the two components. However, in mixed stand with wheat, standard errors of pea fraction yields were considerably smaller in the GM consisting of Balltrap-Dove as compared to the standard errors of the fraction yields of single cultivars and considerably smaller than one of the two components (cv. Dukat) for the GM of Partner-Dukat, giving an indication of a potentially stabilizing effect for pea fraction yields of



GMs. Pea is especially exposed to the strong competitive effect of barley for N at early growth stages, when nodules are not yet formed and both plants compete for available soil N (Bedoussac and Justes, 2010a; Corre-Hellou *et al.*, 2006b). The availability of mineral soil-N to peas in the first five weeks of development greatly determines the leaf area that plants develop in this phase and, as a consequence, the pea plants' capacity for subsequent fixation of atmospheric N due to a greater availability of assimilates (Mahon and Child, 1979). Results from Functional-structural plant modeling (FSPM) further suggest the importance of early dominance in aboveground biomass to maintain competitiveness against the cereal companion (Barillot *et al.*, 2014). As discussed in the previous section, Pr effects of pea – and consequently their GMA – should be increased in order to increase GMA, however, not by selecting longer plants due to the risk of severe lodging. Instead, other strategies to increase pea productivity within mixed stand should be developed. Since only 5% of photosynthetically usable light (400-700nm wavelength) is transmitted through most plant leaves, a large LAI in the top layers of the canopy is of key importance to attain a high assimilation rate for a specific plant species within a mixed plant community (Caldwell, 1987). In order to increase pea-LAI in the crucial top layers of the canopy, while not increasing plant length, three different strategies can be followed: a) selecting for higher plant-stature b) selecting for fully leaved plants or c) selecting for increased stipule leaf area in semi-leafless plants as a Canadian study has shown a correlation of leaf area index (LAI), measured by stipule area, correlated positively with pea yield in mixtures (Jacob *et al.*, 2016). LAI came along with a negative correlation with companion crop yield, however significant only at one out of two locations. The same study reports correlations between plant biomass and pea fraction yield and plant height and pea fraction yield, these however to a stronger negative impact on companion crop fraction yield than stipule area/LAI.

The goals of this study were thus (1) to elucidate the absolute and relative sizes of GMA and SMA variance in a pea-barley mixed crop; (2) to investigate the correlation between pure stand yields in pea and total mixture yields as well as pea fraction yields in mixture; (3) to explore the impact of genotypes on mixture performance, e.g. to determine the GMA, Pr and As effects of genotypes and their stability across environments; (4) To investigate the role of key traits, based on prior knowledge (early vigor, plant height, stipule area, leafiness and biomass) or to mine new traits as potential predictors for GMA. In order to clarify these questions, a multi-year, multi-site field experiment was conducted, using an incomplete factorial design approach (Haug *et al.*, 2021). By putting a special weight on pea, and in order to estimate its GMA and SMA with sufficient precision, a higher number of pea genotypes (27) than barley genotypes (8) were used. The results of this study will help to further advance breeding for mixed cropping in general and breeding of pea and barley for mixed cropping in specific. Our research will thus contribute to the advancement of Agroecological methodology and contribute to its applicability.

## 4.2. Materials and methods

For a description of the plant material, site, soil and management data, please refer to the section “Experimental setup and methodology, Experiment 1 (2018 and 2019)”, page 32.

### 4.2.1. Analysis of yield parameters: GMA, Pr/As, ratio pea, (p)LER and pure stand yield

#### 4.2.1.1. Estimation of variance components for GMA, Pr- and As effects

In order to estimate variance components, the following models, (11), (12) and (13), were applied in which capital letters indicate random effects.

$$y_{ijklm} = \mu + a_k + l_l + r_{m(kl)} + G_{p_i} + G_{b_j} + S_{ij} + (G_p a)_{ik} + (G_p l)_{il} + (G_b a)_{jk} + (G_b l)_{jl} + (S a)_{ijk} + (S l)_{ijl} + E_{ijklm} \quad (11)$$

with  $y_{ijklm}$  the total mixture yield of the *i*-th pea cultivar mixed with the *j*-th barley cultivar in *k*-th year, the *l*-th location and the *m*-th block,  $\mu$  the intercept of mixture yields,  $a$  the effect of the *k*-th year,  $l$  the effect of the *l*-th location,  $r$  the effect of the *m*-th replication nested in year and location,  $G_{p_i}$  and  $G_{b_j}$  the GMA effects of the *i*-th pea cultivar and the *j*-th barley cultivar, respectively,  $S_{ij}$  the SMA effect, i.e. interaction, of the *i*-th pea cultivar with the *j*-th barley cultivar and  $E_{ijklm}$  the error term. The four-way interaction of SMA (GMA x GMA) x year x location was left out for means of simplicity as well as the three-fold interactions between GMA x year x location (little or no influence on total variance, data not shown). The random effects were assumed to be independent and normally distributed with respective variances  $\sigma_{G_p}^2, \sigma_{G_b}^2, \sigma_S^2$  and  $\sigma_E^2$  for GMA of pea and barley, SMA and the error term, respectively and mean zero. The models for each pea and barley fraction yields for all locations and all years were:

$$y_{p_{ijklm}} = \mu_p + a_k + l_l + r_{m(kl)} + P_{p_i} + A_{b_j} + S_{p_{ij}} + (P_p a)_{ik} + (P_p l)_{il} + (A_b a)_{jk} + (A_b l)_{jl} + (S_p a)_{ijk} + (S_p l)_{ijl} + E_{p_{ijklm}} \quad (12)$$

$$y_{b_{ijklm}} = \mu_b + a_k + l_l + r_{m(kl)} + P_{b_j} + A_{p_i} + S_{b_{ij}} + (P_b a)_{jk} + (P_b l)_{jl} + (A_p a)_{ik} + (A_p l)_{il} + (S_b a)_{ijk} + (S_b l)_{ijl} + E_{b_{ijklm}} \quad (13)$$

with  $y_{p_{ijklm}}$  the pea fraction yield of the *i*-th pea cultivar mixed with the *j*-th barley cultivar in *k*-th year, the *l*-th location and the *m*-th block;  $\mu_p$  the intercept of pea fraction yields,  $a_p$  the effect of the *k*-th year,  $l_p$  the effect of the *l*-th location,  $r_p$  the effect of the *m*-th replication nested in year and location on pea fraction yield;  $P_{p_i}$  and  $A_{b_j}$  the Pr effects of the *i*-th pea cultivar and the As effect of the *j*-th barley cultivar, respectively,  $S_{p_{ij}}$  the interaction of the *i*-th pea and the *j*-th barley, and  $E_{p_{ijklm}}$  the error term. Parameters apply in analogy for barley fraction yields in formula 1b. All random effects are assumed to be independent and normally distributed with according variances  $\sigma_{P_p}^2, \sigma_{P_b}^2, \sigma_{A_p}^2, \sigma_{A_b}^2, \sigma_{S_p}^2, \sigma_{S_b}^2, \sigma_{E_p}^2$  and  $\sigma_{E_b}^2$  for Pr-effect of pea and barley, As-effect of pea and barley, SMAs of pea and barley fraction yields and error for pea and barley

fraction yields, respectively, and mean zero. All analysis were done with GNU R (R Core Team, 2019). Mixed model analysis was done with the package "lme4" (Bates *et al.*, 2015) and variance components were extracted for each model.

#### 4.2.1.2. Estimation of broad sense heritability

Broad sense heritabilities on mixture-mean basis of yields and of key-traits were calculated using the standard formula assuming a balanced design (Schmidt *et al.*, 2019), using the variance components obtained by the models given in equations (11), (12) and (13):

$$H^2 = \frac{\sigma_G^2 + \sigma_S^2}{\sigma_G^2 + \sigma_S^2 + \frac{\sigma_{Gl}^2}{2} + \frac{\sigma_{Sl}^2}{2} + \frac{\sigma_{Ga}^2}{2} + \frac{\sigma_{Sa}^2}{2} + \frac{\sigma_E^2}{8}} \quad (14)$$

With  $\sigma_G^2$  the cumulated variance components of pea and barley cultivars (GMAs in the case of total yield, Pr- and As-effects in the case of fraction yields),  $\sigma_S^2$  the variance component of SMA,  $\sigma_{Gl}^2$ ,  $\sigma_{Ga}^2$ , the cumulated variance components of the pea and barley treatment x location and pea and barley treatment x year interaction, respectively,  $\sigma_{Sl}^2$ ,  $\sigma_{Sa}^2$  the variance components of the pea x barley x location and year interactions (SMA x location, SMA x year) and  $\sigma_E^2$  the variance component of the error. The denominator numbers correspond to the number of locations (2), years (2) and total plots (locations x years x replicates = 8), respectively.

#### 4.2.1.3. Comparison of pure stand yields with total mixture yields and pea fraction yields of pea cultivars

Correlations between pure stand yields of pea and total mixture yield and pea fraction-yield were used as a proxy for the predictive power of pure stand yields for these parameters. The data is presented as a scatterplot in Fig. 10, p. 63. Spearman-correlations were calculated once with all genotypes involved ('rho') and once omitting all leafy genotypes and the mixture containing leafy genotypes (GM-long, 'r'). These genotypes represent a distinct group within the pea treatments, having a poor pure stand yield due to strong lodging.

#### 4.2.1.4. Estimation of genotype-wise effects for GMA, Pr- and As-effects, pea-ratio in mixture and pure stand yields

Simplified versions of models in formulas (11), (12) and (13) without SMA and setting all effects as fixed, were used to compute the effects of pea and barley cultivars on yield parameters (GMA, Pr and As effects) across experiments (Table 6 left, p. 66) using estimated marginal means (Lenth, 2019). The same simplified version of the model in formula (11) was used to estimate marginal means of pea and barley cultivars for pea ratio in total yield, LER and pLER, and pure stand yields (Table 6 right, p. 66). The ratio of pea in total yield indicates the share of pea fraction yield by the total mixture yield of each plot. Land equivalent ratio (LER) and partial land equivalent ratios (pLERs) were calculated on a per plot basis according to Mead and Willey (1980). The pLERs were calculated dividing the fraction yields of a mixture in each plot by the mean pure stand yield of the corresponding pea and barley cultivars at a given experiment. For example for an arbitrary

pea cultivar  $i$ , the formula is  $pLER_{p_i} = y_{p_{i_{mix}}} / y_{p_{i_{pure}}}$ . The pLER of barley cultivar  $j$  was calculated similarly:  $pLER_{b_j} = y_{b_{j_{mix}}} / y_{b_{j_{pure}}}$ . Consequently, the LER of a plot with the mixture of pea cultivar  $i$  and barley cultivar  $j$ ,  $LER_{ij}$ , was then calculated by the sum of both partial LERs:  $LER_{ij} = pLER_{p_i} + pLER_{b_j}$ . For pure stand yields also the model in formula (11) was used, however all effects of the other species, and interactions with it were omitted. GMA, Pr and As effects are measured in the same unit as yield, i.e. in t/ha. Within each species, all cultivar-effects were tested against the  $H_0$  of having no (zero) effect using a t-test with a p-value adjustment for multiple testing using the false-discovery-rate procedure of Benjamini and Hochberg (1995). Next to the GMA, Pr- and As-effects, the sum of genotype x experiment interactions of each genotype (ecovalence,  $W_i$ ) was computed as a measure of variability of genotypes' effects across experiments (Becker and Leon, 1988; Wricke, 1962). This calculation was done using the `ge_stats` function of the GNU R package 'metan' (Olivoto and Lúcio, 2020) regarding each of the four experiments as a separate environment and thus quantifying the GxE interaction of each genotype on this basis.

#### 4.2.1.5. Graphical analysis of GMA, Pr- and As-effects

Pr- and As-effects were plotted as a scatterplot and color-coded by their plant type (Fig. 12). Plant type was described by the leaf type (leafy or semi-leafless) and using the *a posteriori* canopy height classification (see 'plant material'). Pr- and As- effects were plotted against GMA (Fig. 12 B and C and Fig. 13 B and C) and Pearson correlations were calculated.

#### 4.2.1.6. Comparison expected and observed yields of GMs

Expected values of GMA, Pr- and As- effects of all four pea GMs were calculated by taking the mean of these effects of all corresponding single cultivars that were contained in the respective mixture (see Table 6). GM-leafy and GM-long both contained P23 (cv. Vitra), an extremely late maturing genotype that was excluded after 2018, however, for means of consistency, was kept in these mixtures for the following year. The 2018 values of P23 were used to calculate expected values of these two mixtures in 2019. Results were displayed graphically (Fig. 14, p. 69).

#### 4.2.1.7. Evaluation of Pr- and As-effects as predictors for yield stability

Scatterplots of the treatments' ecovalence of their Pr-effects and the ecovalence of their GMA was created and a correlation analysis was done (idem for As-effects; see Fig. 11, p. 66).

### 4.2.2. Key trait analysis

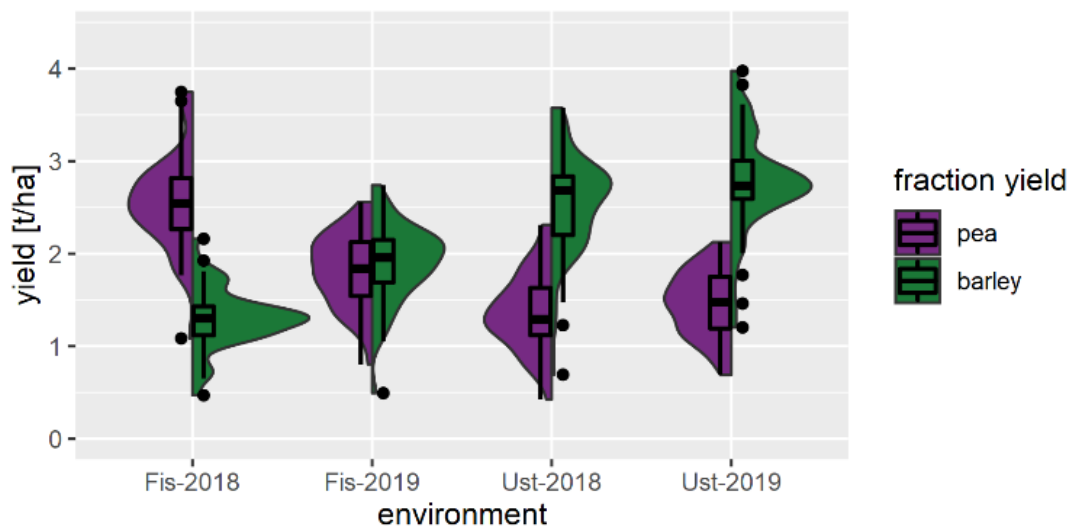
Before picking meaningful key-traits for further analysis, multicollinearity among traits was assessed. A trait-selection procedure was applied in which each trait was checked for its correlation with the other traits. If a trait was correlated with a coefficient of 0.65 or more with one or several other traits, among these multicollinear traits, the one with the highest correlation with total mixture yield (and at least a correlation of 0.3 with mixture yield) was selected for further analysis and the others were discarded (Fig. A8, p. 94). Using this method, early vigor,

onset of flowering, plant biomass and stipule length were selected for further analysis. These traits were plotted against GMA, Pr- and As effects and their correlations computed (Fig. 15, p. 70) The GMs were excluded from the trait analysis for inconsistent measurements due to their heterogeneity and P28 (cv. Florida) was excluded from this analysis since it represents a very distinct genotype, having an extreme biomass production, being fully leaved and having a strong tendency to lodge. Biomass had a high correlation with pea canopy height at grain filling (data not shown) and thus biomass will be treated as proxy for plant length throughout the manuscript.

### 4.3. Results

#### 4.3.1. Sizeable GMA, Pr- and As-effects and negligible SMA effects of pea and barley genotypes

The four experimental environments differed considerably in terms of climate with +11.1% and -3.5% temperature sums and -31.1% and -24.7% cumulative rainfall for 2018 and 2019, respectively, compared to the long-term average (Fig. A3, p. 91). Soil nutrient status and composition also differed between sites with in average 8% higher sand and 16% lower clay content (of total soil particles) at the Fislisbach site compared with the Uster site (Table A4, p. 92). Also soil mineral nitrogen levels were generally lower (-64%) at Fislisbach compared to Uster (39kg N/ha mean Fis vs. 106kg N/ha mean Ust). The mean total mixture yield across all experiments was 3.9t/ha. Plants responded to the variable conditions with a min-max range for pea and barley fraction yields across all four experimental environments of 1.25 t/ha and 1.43 t/ha, respectively (Fig. 8), and of 0.55t/ha for total mixture yield.



**Fig. 8.** Overview over yield data (plot level) of the four experimental environments. The locations Fislisbach and Uster are abbreviated 'Fis' and 'Ust', respectively. (For pure stand yields see Fig. A12, p. 98).

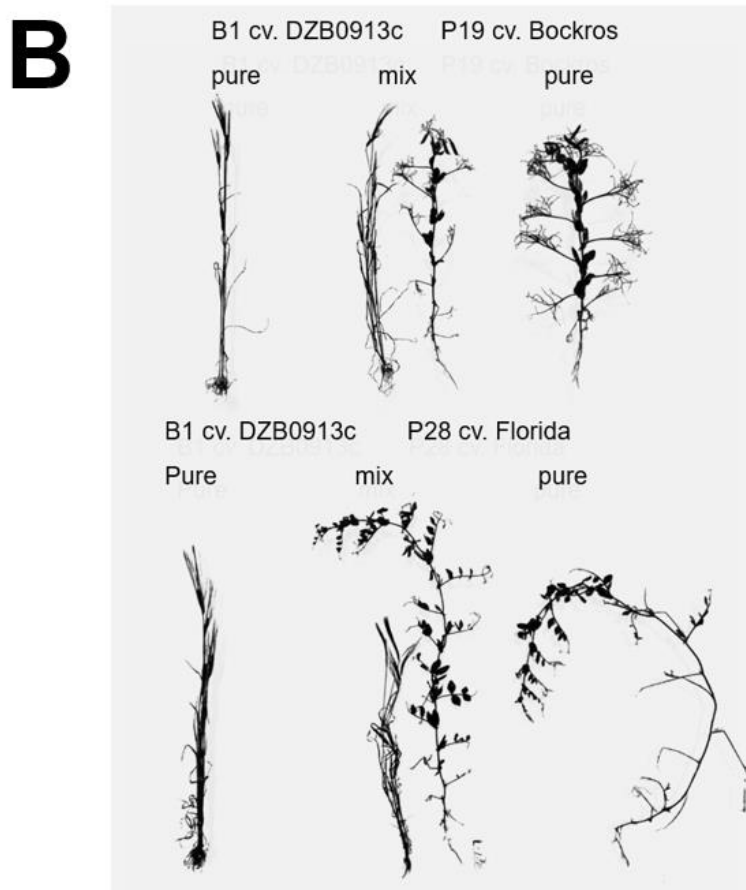
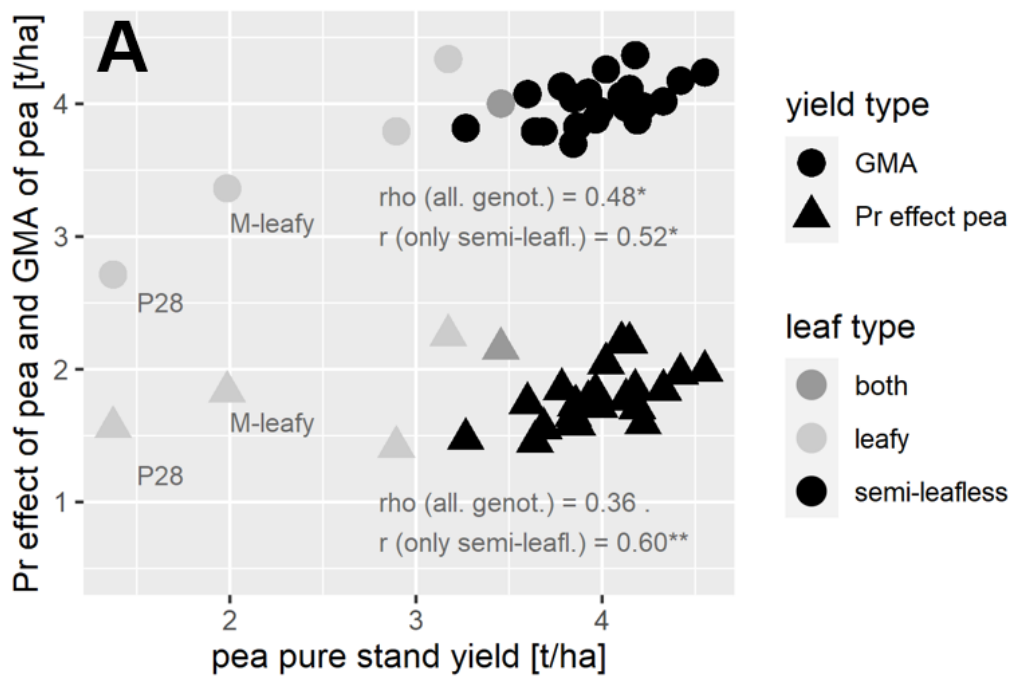


**Fig. 9.** Size of variance components of pea and barley genotype and their interaction with each other or the environment. In the case of pea fraction yield, pea genotype ('pea gen.') corresponds to Pr (producer) effect of pea and barley genotype ('barley gen.') to As (associate) effect of barley. Consequently, for barley fraction yield, pea genotype corresponds to As effect of pea and barley genotype to Pr effect of barley. For total yield, pea genotype and barley genotype correspond to the GMA of pea and barley. For all yield types pea genotype x barley genotype corresponds to the specific SMAs.

Pea fraction yield was influenced by pea genotype (Pr pea) x year interactions and, to a smaller degree, by pea genotype (Pr pea), pea genotype (Pr pea) x location interactions and barley genotype (As barley). Broad sense heritability ( $H^2$ ) of pea fraction yield was 0.37. Barley fraction yield was influenced by pea genotype (As), barley genotype (Pr) and – to a lower degree – by the year and location interactions of pea and barley genotypes. The leafy genotypes of pea were the main reason causing the large variance component for pea genotype (As pea) on barley yield (data not shown). Broad sense heritability  $H^2$  for barley fraction yield was 0.75. Variance components of SMA and its interactions were zero for both pea and barley fraction yields. Total mixture yield was mainly influenced by pea genotype (GMA pea), pea genotype (GMA pea) x year interactions, and barley genotype (GMA barley) x location interactions (Fig. 9). Variance components of SMA and its interactions with year and location were zero or close to zero. Broad sense heritability ( $H^2$ ) of total mixture yield was 0.59.

### 4.3.2. Correlation of pea GMA and Pr-effects with pure stand yields associated to a single trait

Correlations of pure stand yields with GMA and Pr effects were dependent on the presence of foliar leaves (and consequently the absence of large tendrils) in pea (Fig. 10 A, following page). All leafy genotypes and GM-long (containing, amongst others, long, leafy genotypes) were strongly lodging in pure stand but only P28 and GM-leafy were also lodging strongly when mixed with barley. Thus, the latter two were seen as outliers and excluded from correlation analysis. If all remaining leafy genotypes, P14, P29, and the GM containing leafy types, GM-long, were analyzed together with semi-leafless genotypes, no significant correlation was observed, neither between pea pure stand yield and GMA ( $r=0.31$ ,  $p=0.123$ ), nor between pea pure stand yield and Pr effect of pea ( $r=0.28$ ,  $p=0.18$ ; Fig. 10 A). However, if only semi-leafless genotypes were observed, significant correlations were observed between pure stand yields and GMA and pure stand yields and Pr effects ( $0.52$ ,  $p=0.013$  and  $0.60$ ,  $p=0.003$ , respectively). Both pea and barley genotypes reacted plastically with altered phenotypes between pure and mixed condition (Fig. 10 B, following page).



**Fig. 10** (A) Scatterplot of GMA and pea Pr effect as a function of pea pure stand yield. Every dot represents one pea genotype. Two correlations were computed per effect, one using all genotypes (Spearman correlation used due to two outlying observations on the left, 'rho') and the other one analyzing only semi-leafless genotypes (black symbols, Pearson correlation, 'r'). (B) Pictures of plants from pure stand and mixture plots of two exemplary combinations (representative plants taken from plots at the Fislisbach 2019 experiment).



### 4.3.3. Genotypes acted and reacted in a multifaceted manner towards mixed cropping

Pea genotypes displayed a larger spread of yield effects than barley genotypes for all relevant yield parameters: GMA, Pr, As, ratio of pea, LER, and pure stand yield (Table 6). For instance, GMA effects of pea ranged from -1.23 to +0.43 t/ha, and from -0.19 to +0.24 t/ha for barley. The average ratio of pea in the total mixture yield was 0.46, hence lower than the one expected due to sowing-ratio (0.66). Average LER across all treatments and experiments was 1.09 with a LER range on a genotype level from 0.99 (P18) to 1.48 (P28). Only leafy genotypes and GM-long (which contained a considerable portion of leafy genotypes) showed significant positive LERs. When these genotypes were excluded and LER was analyzed only among semi-leafless genotypes, the only genotype showing a significant effect on LER was P20 ( $p=0.043$ ; by stimulating an above average pLER in barley, pLER data not shown). Three genotypes with negative and four genotypes with a positive GMA, were observed. Among those with negative GMA, lodging was the reason in two out of three cases (P28 and GM-leafy). Among those with a positive GMA, a broad spectrum of different trait- and effect-patterns was associated with their positive effect on mixture yield: for example while for P20, the above average GMA was associated with a positive As-effect and earliness (Table 6, Table A9, p. 94), the positive GMA of P14 was due to a high, positive Pr effect that wasn't offset by an equally strong negative As effect. A genotype mixture (GM), GM-semi-leafless, was the most stable genotype in terms of GMA across all experiments ( $W_i = 0.004$ ). Another GM, GM-leafy, was the most stable genotype regarding Pr effects ( $W_i = 0.04$ ). Interestingly, while their mixture was the most stable genotype in terms of Pr effects, the three individual leafy genotypes P14, P28 and P29 were the three most unstable genotypes of the whole set of genotypes regarding Pr effects, with P14 being the most unstable ( $W_i = 1.97$ ); the three leafy types were also the three most unstable genotypes in terms of GMA and also exhibited below average stability for As effects ( $W_i = 0.48$  and  $W_i = 0.54$ , respectively). Among barleys, four genotypes had a GMA that was significantly different from 0. The barley genotypes' GMA was positively correlated with the barley genotypes' pure stand yield (Fig. A10, p. 96). Stability of individual barley genotypes for the different yield parameters (expressed by  $W_i$  for GMA, Pr and As effects, influence on ratio of pea and pure stand yields) was – on average – considerably higher than for pea genotypes.

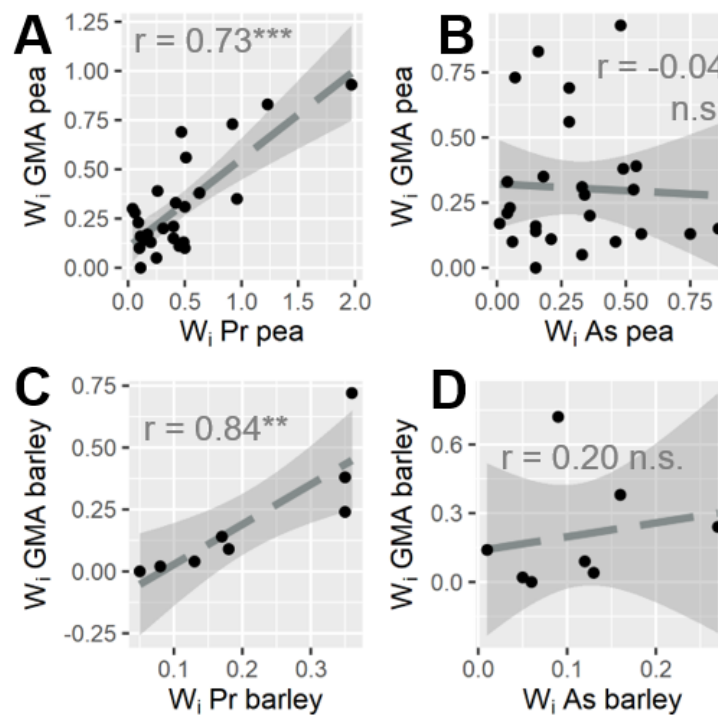
Pea genotype	Plant size and leafiness <sup>1</sup>		Total yield		Pea fraction yield		Barley fraction yield		Ratio pea of total yield	LER	Pure stand yield pea		
			GMA pea [t/ha]	W <sub>i</sub>	Pr-effect pea [t/ha]	W <sub>i</sub>	As-effect pea [t/ha]	W <sub>i</sub>			W <sub>i</sub>	W <sub>i</sub>	Pure stand yield pea [t/ha]
P01-SG-L 7647	L	s. leafl.	-0.24	0.69	-0.14	0.47	-0.11	0.28	0.00	0.012	-0.09 *	0.12	0.60
P02-Impuls	L	s. leafl.	0.17	0.10	0.41 **	0.50	-0.22	0.46	0.09 **	0.030	-0.03	0.43 *	0.98
P03-Astronaute	S	s. leafl.	0.03	0.33	-0.02	0.42	0.05	0.04	-0.02	0.010	-0.08	0.41 *	0.45
P05-Navarro	S	s. leafl.	0.01	0.28	-0.08	0.06	0.08	0.34	-0.01	0.003	-0.06	0.27	0.39
P06-Gambit	L	s. leafl.	0.12	0.31	0.41 **	0.50	-0.30 *	0.33	0.10 ***	0.014	-0.09 *	0.38 *	0.46
P07-Angelus	S	s. leafl.	-0.15	0.13	-0.23	0.20	0.08	0.56	-0.04	0.017	-0.08	-0.03	0.23
P08-Salamanca	L	s. leafl.	0.23	0.20	0.17	0.31	0.11	0.36	0.00	0.017	-0.05	0.70 ***	0.54
P10-Rocket	M	s. leafl.	0.04	0.38	-0.20	0.63	0.23	0.49	-0.06 *	0.038	-0.06	0.50 **	0.71
P11-Karpate	S	s. leafl.	0.11	0.21	-0.06	0.40	0.17	0.04	-0.03	0.011	-0.01	0.14	0.36
P12-Kayanne	M	s. leafl.	0.32 *	0.17	0.26	0.17	0.09	0.01	0.01	0.003	0.01	0.30	0.75
P13-Mytic	S	s. leafl.	-0.11	0.73	-0.21	0.92	0.11	0.07	-0.04	0.031	-0.06	0.15	2.48
P14-Protecta	L	leafy	0.40 ***	0.93	0.47 ***	1.97	-0.07	0.48	0.06 *	0.049	0.15 ***	-0.55 **	1.12
P18-Alvesta	S	s. leafl.	-0.07	0.16	-0.08	0.11	0.00	0.15	-0.01	0.003	-0.10 *	0.47 *	1.64
P19-Bockros	M	s. leafl.	0.10	0.56	-0.19	0.51	0.25	0.28	-0.06	0.011	-0.03	0.12	2.60
P20-Volt	M	s. leafl.	0.43 ***	0.13	0.06	0.49	0.37 **	0.75	-0.03	0.032	0.04	0.46 *	1.26
P21-Biathlon	S	s. leafl.	0.13	0.11	-0.04	0.45	0.18	0.21	-0.02	0.024	0.03	-0.12	0.24
P22-Tip	M	s. leafl.	-0.06	0.15	0.03	0.40	-0.10	0.86	0.02	0.031	-0.07	0.25	0.11
P24-Peps	L	s. leafl.	0.08	0.35	0.05	0.96	0.02	0.18	0.00	0.040	-0.08	0.61 **	0.65
P25-Karioka	M	s. leafl.	0.30 *	0.05	0.20	0.25	0.10	0.33	0.02	0.015	-0.05	0.83 ***	1.26
P27-Milwa	S	s. leafl.	-0.12	0.10	-0.32 *	0.10	0.19	0.06	-0.07 **	0.007	0.02	-0.45 **	1.54
P28-Florida	L	leafy	-1.23 ***	2.40	-0.23	1.49	-1.00 ***	0.54	0.11 ***	0.020	0.39 ***	-2.35 ***	0.59
P29-Natura	S	leafy	-0.15	0.83	-0.37 **	1.23	0.22	0.16	-0.09 **	0.039	0.03	-0.83 ***	3.12
P31-Starter	S	s. leafl.	-0.15	0.39	-0.33 *	0.26	0.19	0.54	-0.08 **	0.019	-0.06	-0.08	0.28
P09-GM-short	M	both	0.14	0.23	-0.03	0.09	0.16	0.05	-0.01	0.002	-0.03	0.21	0.67
P26-GM-long	L	both	0.06	0.14	0.37 **	0.14	-0.31 *	0.15	0.09 **	0.004	0.03	-0.26	0.38
P04-GM-semi-leafless	M	s. leafl.	0.19	0.00	0.06	0.11	0.13	0.15	0.00	0.006	0.02	0.06	0.43
P32-GM-leafy	L	leafy	-0.58 ***	0.30	0.04	0.04	-0.62 ***	0.53	0.11 ***	0.020	0.33 ***	-1.73 ***	2.00
min. (effect)			-1.23	0.00	-0.37	0.04	-1.00	0.01	-0.09	0.002	-0.10	-2.35	0.11
mean (effect)			0.00		0.00		0.00		0.00		0.00	0.00	
max. (effect)			0.43	2.40	0.47	1.97	0.37	0.86	0.11	0.049	0.39	0.83	3.12
<b>mean</b>			<b>3.94</b>	0.38	<b>1.79</b>	0.49	<b>2.14</b>	0.31	<b>0.46</b>	0.019	<b>1.09</b>	<b>3.72 (pea)</b>	0.96

Barley genotype	Competition score <sup>2</sup>	GMA barley		As effect barley		Pr effect barley		Ratio pea of total yield	LER	Pure stand yield barley			
		[t/ha]	W <sub>i</sub>	[t/ha]	W <sub>i</sub>	[t/ha]	W <sub>i</sub>			[t/ha]	W <sub>i</sub>		
B1-DZB0913c	9	0.14 *	0.24	-0.22 **	0.27	0.37 ***	0.35	-0.07 ***	0.016	-0.01	0.34 *	1.08	
B2-Eunova	8	0.24 *	0.72	-0.19	0.09	0.43 ***	0.36	-0.08 ***	0.001	0.02	0.23	0.30	
B3-GM-barley	6	-0.07	0.02	-0.07	0.05	-0.01	0.08	-0.01	0.004	0.02	-0.29 *	0.46	
B4-Zeppelin	1	-0.02	0.00	0.15	0.06	-0.18	0.05	0.05 *	0.001	-0.03	0.15	0.32	
B5-KWS Atrika	5	0.11	0.14	0.14	0.01	-0.03	0.17	0.02	0.004	-0.02	0.30 *	0.21	
B6-Propino	4	-0.19 *	0.38	0.09	0.16	-0.28 ***	0.35	0.03	0.007	0.05	-0.70 ***	0.26	
B7-Rubaszek	5	-0.19 *	0.04	0.08	0.13	-0.27 ***	0.13	0.04 *	0.008	-0.08 **	0.20	0.44	
B8-KWS Irina	6	-0.01	0.09	0.02	0.12	-0.04	0.18	0.01	0.008	0.04	-0.23	0.32	
min. (effect)			-0.19	0.00	-0.22	0.01	-0.28	0.05	-0.08	0.001	-0.08	-0.70	0.21
mean (effect)			0.00		0.00		0.00		0.00		0.00	0.00	
max. (effect)			0.24	0.72	0.15	0.27	0.43	0.36	0.05	0.049	0.05	0.34	1.08
<b>mean</b>			<b>3.94</b>	0.20	<b>1.79</b>	0.11	<b>2.14</b>	0.21	<b>0.46</b>	0.016	<b>1.09</b>	<b>3.78 (barley)</b>	0.42

<sup>1</sup>Plant sizes: S(hort), M(edium) and L(ong); leafiness: semi-leafless ('s. leafl.') or 'leafy', <sup>2</sup>Ranging from low, i.e. '1', to high, i.e. '9', competition, see main text

**Table 6. (See previous page).** Total mixture yield, pea and barley fraction yield as well as pure stand yield, along with ratio of pea in total yield and land equivalent ratios (LERs) for the 27 pea and 8 barley genotypes used in the trial. All variables are expressed as effects, i.e., centered around the average of each variable within each species over all blocks, locations and years. Significances are given for a t-test against the mean (i.e., against 'zero' in this case where effects are centered around the average); Next to the GMA, Pr- and As-effects, and pure stand yield, the genotype x experiment interaction of each genotype is given in the form of ecovalence ( $W_i$ ) with higher values indicating stronger GxE interaction.

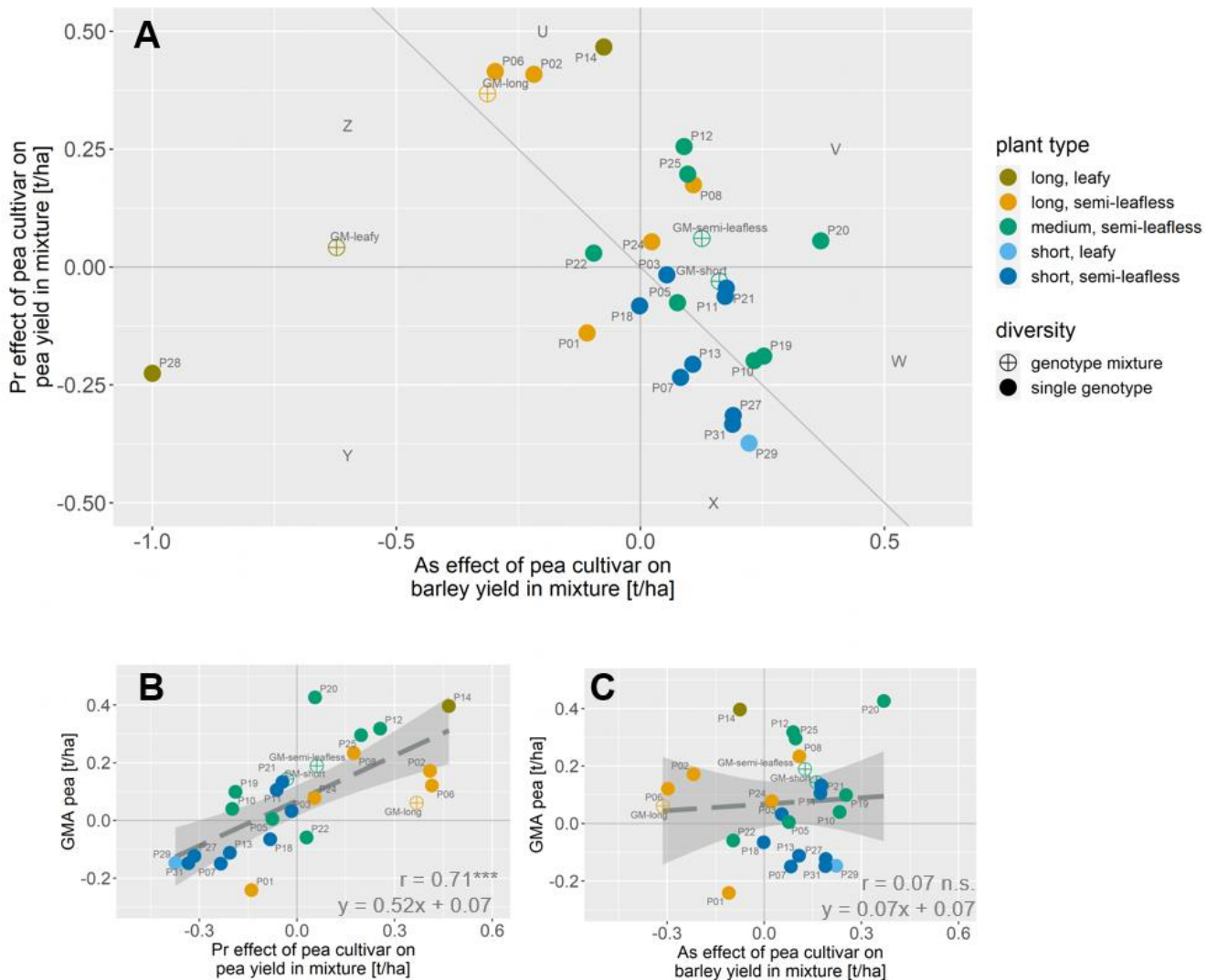
Ecovalence of GMA was correlated positively with the  $W_i$  of the Pr effects and was uncorrelated with the  $W_i$  of As effects in both pea and barley (Fig. 11). This major observation was made for both species, pea and barley (see Fig. A13A and B, p. 99, for a correlation matrix of stability of GMA, Pr- and As-effects and pure stand yields).



**Fig. 11.** Scatterplots of Ecovalence ( $W_i$ ) of Pr- and As- effects of pea and barley with the  $W_i$  of their corresponding GMA.

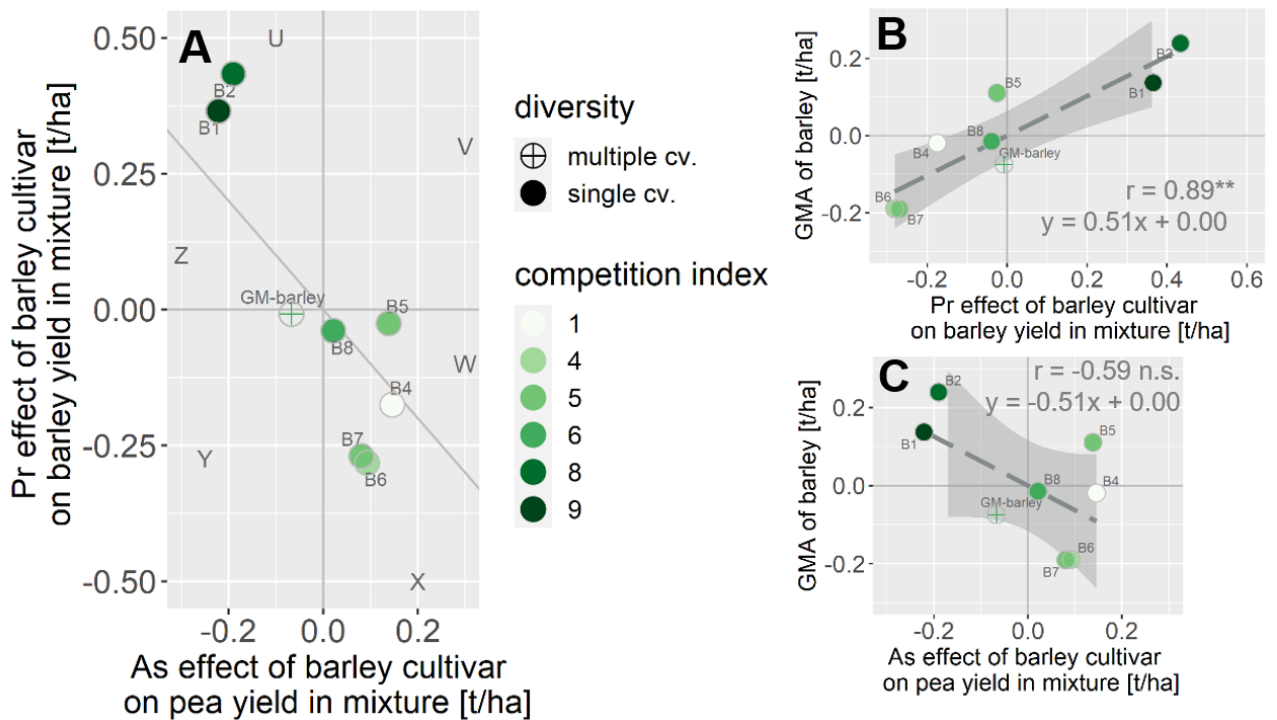
A negative relationship was observed between Pr and As effects, reflecting a tradeoff between these two effects (Fig. 12 A). The regression-analysis between these two parameters yielded a function of  $Pr = -0.93 \cdot As$  and a correlation of  $-0.65$  ( $p=0.0004$ ). The two genotypes P28 and GM-leafy were omitted for this purpose due to their exceptionally high lodging in mixture. Seven genotypes were observed to break with the tradeoff-pattern between Pr- and As-effects, having a significant positive (the medium or long genotypes P12, P14, P20, P25) or negative (P01, P28, GM-leafy) GMA (Fig. 12 A, Table 6). Among the four genotypes with positive GMA effects, three show a dominant Pr effect. Medium-sized genotypes exhibited consistently positive GMA (mapping to sectors V and W). Short-sized genotypes did not exhibit a clear pattern regarding GMA (positive or negative). Long sized genotypes, either leafy or semi-leafless, exhibited a wide range of positive or negative As effects and were spread mostly in sectors with positive Pr effects (Z, U and V).

Due to the small number of genotypes (3), no consistent pattern regarding leafiness could be observed. When the two highly lodging genotypes P32 and GM-leafy were left out, GMA of pea was positively correlated with Pr-effects and uncorrelated with As-effects (Fig. 12 B and C).



**Fig. 12.** (A) Pr- and As-effects of 27 pea genotypes over two years and two locations. The line with slope  $-1$  and intercept  $0$  separates genotypes with positive (above) and negative (below) GMA. According to their positive or negative Pr, As or GMA effects, genotypes group into different sectors (U to Z). The regression was  $Pr = 0.93 \cdot As$  with a correlation of  $-0.65$  ( $p=0.0004$ ; the two outliers on the left were excluded, see main text for details). (B) Plot of Pr-effects and GMA of pea. (C) Plot of As-effects and GMA of pea. The strongly lodging genotypes P28 and P32 were excluded in the latter two plots. Dashed lines indicate the corresponding regression between Pr-/As-effects and GMA with 95% confidence-intervals.

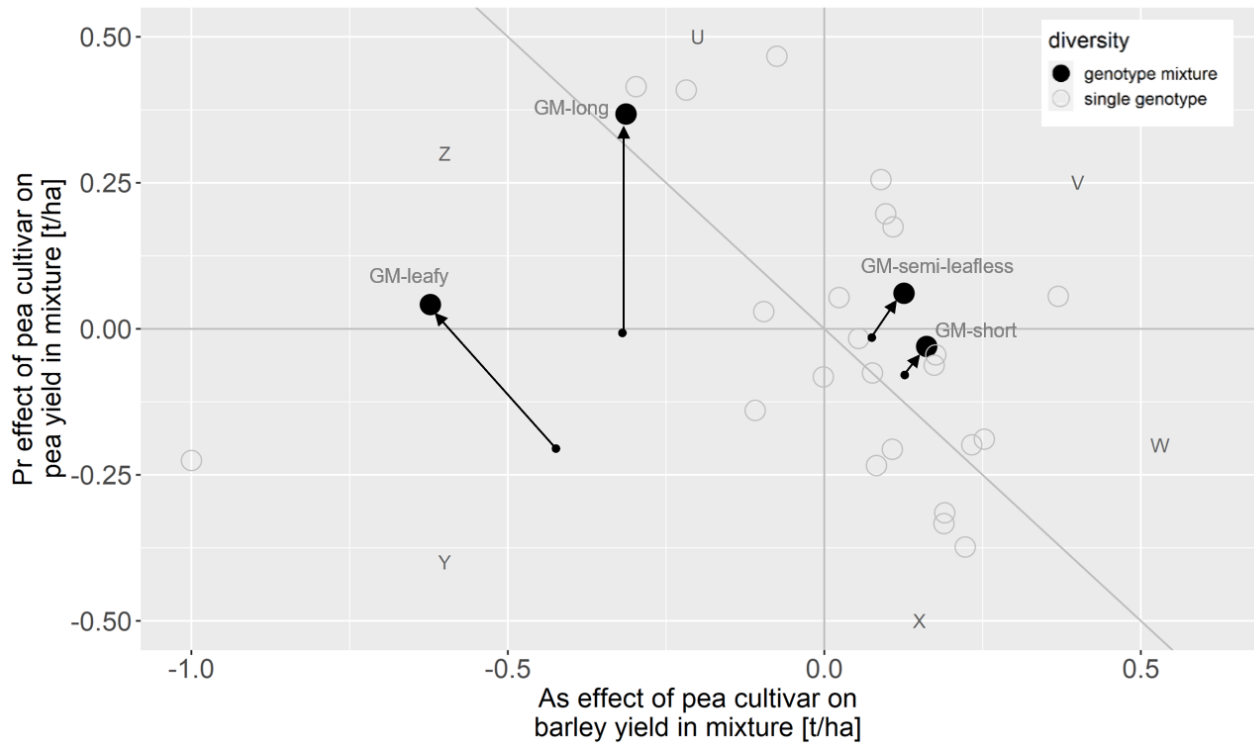
Like for pea, a negative trend between Pr- and As-effects was observed for barley with a regression-function of  $Pr = -1.65 \cdot As$  and a correlation of  $-0.89$  ( $p=0.0034$ , Fig. 13 A, following page; as GMA, Pr and As are all effects, meaning being centered around zero, the intercept is always zero when plotting against each other). Four genotypes displayed significant effects for GMA in barley, two positive (the high competition index genotypes B1, B2) and two negative (B6, B7; see Table 6). As for pea, barley GMA was positively correlated with Pr-effects (Fig. 13B) and the correlation between GMA and As effects was not significant ( $p=0.13$ ; Fig. 13C).



**Fig. 13.** (A) Pr- and As-effects of eight barley genotypes over two locations and two years. The line with slope  $-1$  and intercept  $0$  separates genotypes with positive (above) and negative (below) GMA. The regression was  $Pr = -1.65 \cdot As$  with a correlation of  $-0.89$  ( $p=0.003$ ). (B) Plot of Pr-effects and GMA of barley and (C) plot of As-effects and GMA of barley. Dashed lines indicate the corresponding regression between Pr-/As-effects and GMA with 95% confidence-intervals.

#### 4.3.4. Genetic heterogeneity in pea genotypes promoted stability in interspecific mixtures of two important yield parameters

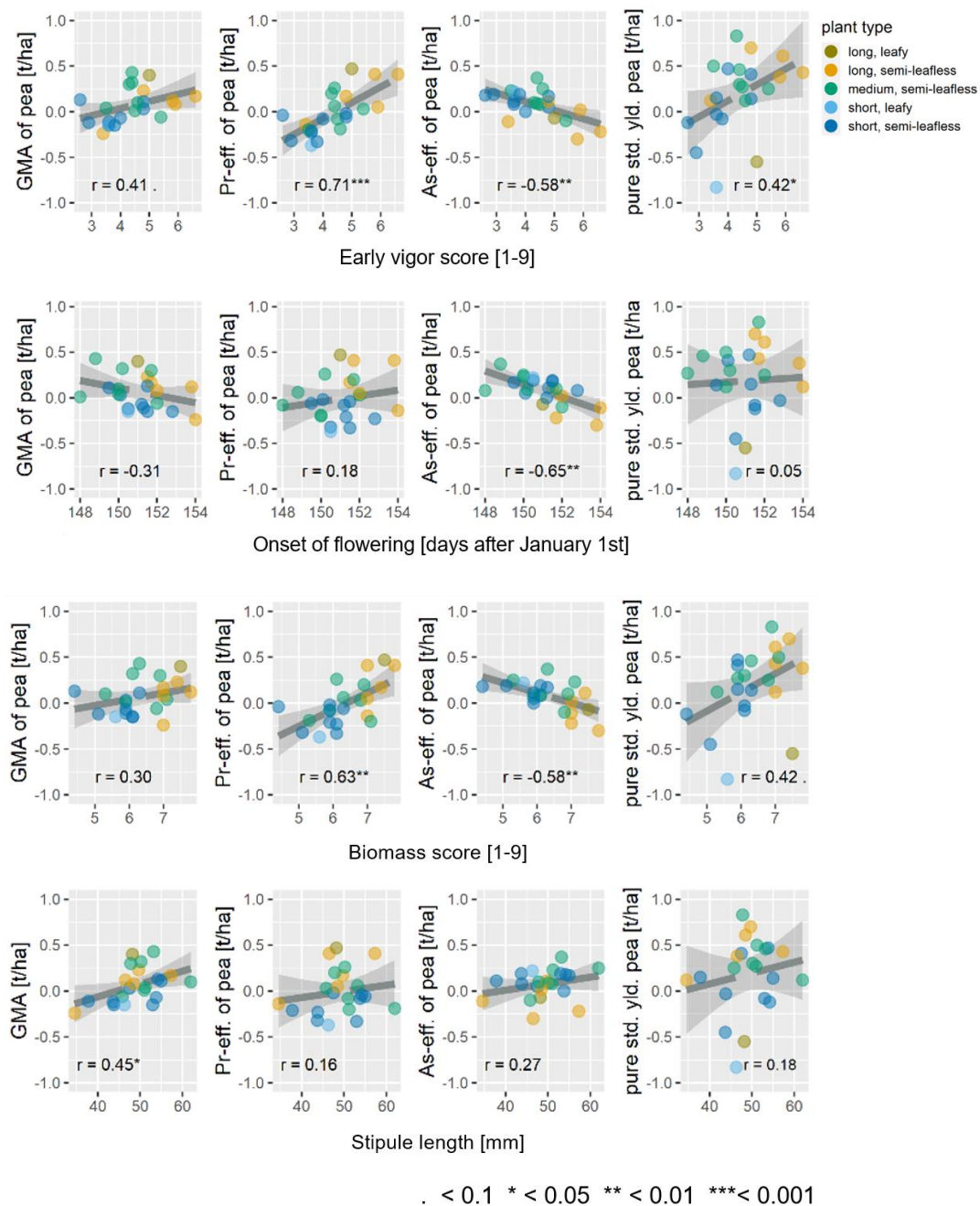
Four out of the eight pea genotypes with the lowest  $W_i$  for Pr effects were four GMs (genotype mixtures; Table 6). For the pea-ratio of total yield, the genotype with the most stable result was a GM (GM-short,  $W_i = 0.002$ ) and among the six genotypes, providing the most stable pea ratios in mixture, were two more GMs (GM-long and GM-semi-leafless). GM-long provided a significantly higher pea ratio (+9%) at a high stability over all four experimental environments ( $W_i$  GM-long =  $0.004 < \text{mean of } W_i 0.049$ , Table 6), as well as a significantly higher GMA compared to expected by its components. Differences in GMA between realized and expected values were for GM-long  $+0.39$  t/ha ( $p=0.040$ ), GM-semi-leafless  $+0.14$  t/ha ( $p=0.203$ ), GM-short  $+0.09$  t/ha ( $p=0.153$ ) and GM-leafy  $-0.05$  t/ha ( $p=0.324$ ). Deviations of Pr- and As-effects (and consequently of GMA) of GMs from the expected values are displayed in Fig. 14.



**Fig. 14.** Expected versus measured values of genotype mixtures (GMs). The small black dots are the expected value of the GM that are based on the arithmetic means of the Pr- and As-effects of the genotypes that are contained in the corresponding GM. The black arrows connect the expected mean of the mixture with the actual mean of the mixture (large black dots).

#### 4.3.5. Analysis of key traits and determination of their biological interaction functions (BIFs)

Broad-sense heritabilities of the four key-traits, early vigor, onset of flowering, biomass, and stipule length were in a medium to high range with values of 0.49, 0.86, 0.71 and 0.89, respectively. Stipule length showed a significant correlation with GMA ( $p=0.034$ ) and early vigor tended towards a significant correlation with GMA ( $p=0.058$ ), while onset of flowering and biomass were not significantly correlated with total mixture yield ( $p=0.160$  and  $p=0.174$ , respectively; see Fig. 15, following page).



**Fig. 15.** GMA, Pr and As effects and pea pure stand yields as functions of four putative pea-key-traits: early vigor (row one), flowering time (row two), biomass (row three) and stipule length (row four). The grey line denotes the linear regression with 95% confidence intervals. GMs were excluded due to heterogeneous trait measurements, and P28 was excluded due to strong lodging.

Concerning traits' biological interaction functions (BIFs; see Haug *et al.*, 2021), early vigor was associated with an antagonistic (opposite slopes in Pr and As regressions) as well as with a commensalistic BIF (one of the two slopes predominates, leading to a positive/negative shift in GMA; in this case Pr predominated and lead to a slight positive effect on GMA). Biomass, however, corresponded to a clear antagonistic trait action among the crops. This means that higher trait values caused a higher pea Pr effect at the cost of lower As effect. Onset of flowering showed a tendency towards an amensalistic trait action: while pea Pr effect was not significantly affected, earlier flowering pea plants were associated with a higher As effect. Stipule length showed a tendency towards a mutualistic or commensalistic trait action: while neither Pr nor As effects were significantly

positively correlated with stipule length, no tradeoff (antagonism) between Pr effect and As effect was observed and its correlation with GMA was positive. Early vigor was associated with higher pure stand yield ( $r = 0.43$ ,  $P = 0.048$ ), and biomass tended to stimulate higher pure stand yields ( $P = 0.051$ ) as well. Neither onset of flowering, nor stipule length were associated with higher pure stand yields.

## 4.4. Discussion

### 4.4.1. Consequences for breeding for mixed cropping

This study investigated the genetic effects of a morphologically diverse panel of 27 pea and 8 barley genotypes on total and fraction yields of their mixed crops in the scope of providing insights for breeding for mixed cropping in general and of pea and barley in specific. Our finding that SMA was low or absent in our scenario simplifies future efforts to select superior genotypes for spring-pea spring-barley mixed crops. Any predictions made by GMA will not be impaired by unpredictable effects due to pea-genotype-by-barley-genotype interactions. This observation of little to zero SMA might be due to the low morphological diversity in the barley genotypes used. It might thus be related to the corn-climbing-bean example (Hoppe, 2016; Starke, 2018), in which also no SMA was observed, which in this case also might be related to the low morphological diversity in German (silage) corn breeding material. On the pea side, morphological diversity was higher, but no landraces or gene-bank accessions were used which would yet have increased diversity. Thus depending on what material is used, especially when specific cultivars for mixed cropping are being developed, whose traits change important parameters such as the leaf area or phenology (see discussion of key traits), SMA should be regularly monitored in future breeding efforts. For the status quo, our results suggest that currently available pea and barley material can readily be combined and genotypes of both species with high GMA are expected to produce best results. The fact that no specific mixing ability (SMA) was observed also facilitates the adoption of spring-pea spring-barley mixtures by farmers as it simplifies the assembly of fitting cultivars. The highest GMA was attained by a leafy genotype and given the small number of genotypes registered, it might be tempting to consider this plant type for breeding for mixed cropping as one might consider it a market opportunity in the context of breeding for mixed cropping. However, the high GxE of these genotypes, observed in our data, is an undesirable feature and such an approach must be taken with caution. Note that leafy genotypes have proven to be well adapted to very dry climates (Armstrong *et al.*, 1994), thus their use for mixed cropping in regions with more continental climate than the Swiss one might still be considered. Under the given pedoclimatic conditions of the experiment (maritime influenced central European-climate) the use of leafy types in genotype mixtures (GMs) for mixed cropping use should be examined further, as one mixture, containing both leafy and semi-leafless genotypes (GM-long), showed significant above expectation results and GMA and high yield stability (see 'use of genotype mixtures').

### 4.4.2. Correlation of mixture and pure stand yields

The correlation observed in this study between pure stand yields of pea and mixture yield ( $r=0.52$ ,



$p < 0.05$ ) allows to use pure stand yield information for several purposes in mixed cropping. It can be usefully integrated into a breeding program for mixed cropping (but for semi-leafless forms only), e.g., the parents of superior offspring for mixed cropping can be selected from genotypes with proven high pure stand yield capacity. For farmers and seed retailers who want to assemble a well performing mixture of pea and barley, information on pure stand yield of the corresponding pea cultivar can be useful. Our results support that pea fraction yield in the mixture can be predicted with even higher accuracy by pure stand performance of a genotype. The correlations of pure stand yields vs. mixture yields are in line with other observations in corn-common bean mixtures ( $r = 0.57$  to  $0.61$  in Hoppe, 2016). This recommendation is valid for semi-leafless cultivars only since one leafy cultivar (P14) and the genotype-mixture GM-long 'broke' the regression (Fig. 10 A). Our observation of a medium correlation ( $r = 0.52$ ) between pure stand and mixture yields lies in the range of previously reported values (Annicchiarico *et al.*, 2019) and neither justifies a completely independent breeding program for mixed cropping, nor will a selection by pure stand yields alone lead to high selection gain in mixed cropping.

### 4.4.3. Stability

#### 4.4.3.1. GxE interactions impacted fraction yield stability

The combination of a very dry season (2018) and sandy soils of the Fislisbach 2018 trial location were generally very favorable for pea growth. The strong GxE interaction of leafy types might be due to their specific reaction to the two climatically quite different years, especially regarding precipitation. As mentioned before, it has been shown that long leafy types have a higher tolerance towards drought stress due to a generally more vigorous rooting system and higher rooting depths, as well as their capacity to change leaf exposition to sunlight, leading to an increased water use efficiency (Armstrong *et al.*, 1994). The variability of ratios on a system level across environments (Fig. 8, p. 60) is also present on an individual genotypic level, where also considerable changes in fraction yields, in a certain year or location, occurred (Fig. A11, p. 97). The use of more diverse genetic material, such as genotype mixtures, has shown potential to mitigate this issue, especially in the pea part of the mixtures.

#### 4.4.3.2. Use of genotype mixtures

To our knowledge, we report for the first time results of a mixed-cropping experiment using more than two genotypes at the intra-specific level, investigating the combined effect of inter- and intraspecific diversification on the performance and stability of an annual mixed crop. By the choice of genotypes for our genotype mixtures (GMs) we tried to make them uniform regarding high or low trait expressions of two potential key-traits (plant stature and leafiness), respectively, while for all other traits, GMs remained very heterogeneous. No assembly rule was applied besides this one. Due to incorrect prior information on some of the contained genotypes, GM-long accidentally contained not only long genotypes, but also medium and short ones and therefore had a large trait-divergence for plant-length. Besides that, it also had a large trait divergence for leafiness (leafy and semi-leafless), and another important trait, lodging resistance (high and low). GM-long thus was one of

the morphologically most diverse mixtures for these three traits. The fact that this particular mixture performed significantly better than expected (compared to the expected mixture performances of all its components) might be caused by a positive effect of trait-divergence within this GM of one or several of these three traits. Besides the significantly higher than expected performance of this particular genotype, the generally higher stability of the GMs for their Pr effects was less surprising, as intra-specific diversity has also been shown to stabilize yields of crop yields in pure stand (Reiss and Drinkwater, 2018). We suspect that the higher stability gained by increasing the diversity at the intra-species level (i.e. genotype mixtures, GMs) in pea is governed by the same stabilizing mechanisms that are at play by increasing diversity at the inter-species level (mixed cropping), i.e. by increased niche complementarity and adaptability (Bedoussac and Justes, 2010a; Zhang *et al.*, 2014) and statistical averaging (Doak *et al.*, 1998). Our observations that intraspecific diversification leads to a stabilizing effect in terms of pea fraction yields are in line with the observations made in other experiments (Carlsson *et al.*, 2017) and might be an important future strategy to maintain high and stable yields of a desired component in a mixture. This stabilizing effect of intra-specific diversity within inter-specific mixtures has also been advocated in natural plant communities, and demonstrated in an experimental study on grasslands (Prieto *et al.*, 2015).

#### 4.4.3.3. GMA is correlated with Pr effects, GMA stability with Pr effect stability

To the best of our knowledge, this is the first paper to analyze the stability of fraction yields in an heterospecific plant community. In present study, Ecovalences of Pr effects of pea were more than twice the size of those of its As effects and Ecovalences of Pr effects of barley were one and a half the size of those of its As effects (Fig. 11 C and D, p. 66), with some outliers with Pr pea > 0.8. For both pea and barley genotypes alike, still a positive relationship between the Ecovalences of Pr and GMA and no relationship between the Ecovalences of As effects and GMA were observed. In other words, GxE of the As effects, i.e., an environment-dependent negative or positive effect of the focal species' genotype on the companion crop, was somehow buffered by a gain or loss in productivity in the focal species' genotype. GxE of Pr effects, however, were not compensated for by the companion species. To date no coherent explanation for this observation could be found and this can be a matter of further research. The analogous observation of a present correlation between actual Pr effects and GMA and an absent relationship between As effects and GMA in both species, pea and barley, was contrary to expectations (Fig. 12 B and C, p. 67, and Fig. 13 B and C, p. 68 ) due to the tradeoff between these two effects within each species (Fig. 12 A, p. 67 and Fig. 13 A, p. 68). The fact that pea was at competitive disadvantage in our experiments is in line with observations earlier made for pea-barley mixtures and it was recommended to strengthen competitive ability of the dominated species in order to maximize mixture yields (Annicchiarico *et al.*, 2019). In a scenario where one species is at competitive disadvantage, a positive relationship between the Pr effect of this species and GMA would be expected, since the capacity of a genotype to increase the fraction yield of itself would in most cases be attained by a higher competitive ability against the dominating species. Consequently, the opposite relationship would be expected for the dominating species in such a scenario: increases in total yield would be attained by genotypes of the dominating species with lower competitive ability (i.e., with mostly lower Pr effects) and thus with genotypes with higher

As effects. Our observations break with this expectation and Pr effects of genotypes of both species are positively correlated with their GMA (Fig. 12 B p. 67, and Fig. 13 B, p. 68). We suspect that species-specific free 'niche space' exists in this cropping system. More precisely, we suspect that in such a scenario, there are resource capacities that are blocked for both species by intraspecific competition in pure stand, but that are available in mixed stand due to less interspecific than intraspecific competition (Annicchiarico *et al.*, 2019). The fact that gains in GMA by higher Pr effects are realized without a tradeoff by a negative relationship between As effects and GMA points towards the commensalistic character of this situation. It indicates that there might be yet unlockable potential in both species to select genotypes that occupy the free niche space in mixture even more effectively and thus realize higher mixture yields that are not at the expense of the companion species. Genotypes that significantly positively deviate from the tradeoff between Pr and As effects, as P12 or P20, can be seen as evidence of such behavior. Following this line of argumentation, ideal genotypes for mixed cropping possess traits that facilitate the occupation of the free niche space by being positively correlated with Pr effects and whose 'Pr-As-balance' is positive, i.e. their positive effect on Pr effects is not or not completely offset by a negative effect on As effects.

#### 4.4.4. Key traits

Of the four key traits that impacted total mixture yield, three traits, early vigor, onset of flowering and stipule length, should be considered as primary targets for breeding for mixed cropping. Other studies in peas have shown a correlation between shoot and root biomass at this stage of growth, where early vigor is usually assessed (BBCH 25-35; Wille *et al.*, 2020) and thus genotypes with high early vigor (i.e., high early shoot biomass) can be assumed to also have above average root-biomass. This fact would allow plants with high early vigor to compete better with barley for soil mineral-N at this crucial early growth stage, which translates in a higher net-assimilation rate for atmospheric N of early vigorous genotypes and thus contributes to an improved niche complementarity in mixed stand with barley of such genotypes (Corre-Hellou *et al.*, 2006b). Our results are in line with the prognoses made from prior experiments (Barillot *et al.*, 2014; Corre-Hellou *et al.*, 2006b) and effectively display the importance of early vigor as a key-trait for improving GMA in pea. In our experimental context, onset of flowering was significantly associated with the As effect on barley yield (with early flowering peas significantly improving barley fraction yields), while not being negatively associated with its Pr effect. This might be evidence for a more complementary resource use pattern for light or other resources, i.e., temporal complementarity, between pea and barley with earlier flowering pea genotypes. Bedoussac and Justes (2010) observed a species dynamic complementary for leaf area index in pea and wheat. Therefore, changes in flowering time of either pea or wheat by using earlier or later flowering genotypes are expected to create temporal overlap for these peak values of leaf area indices and thus to change the light use efficiency of the total mixed crop. Nothing is known on the specific temporal dynamics on light use efficiency of spring-pea spring-barley intercrops and our suggested explanation for the observed phenomenon has yet to be verified by measurements of temporal leaf area partition between the two crops. When it comes to actual leaf area, three strategies can be proposed to increase pea LAI in the top layers of

the canopy of mixed crops: a) higher plant stature, b) leafy genotypes c) increasing stipule area. Strategy a), higher plant stature, is not recommended (see following paragraph). As for strategy b), leafy genotypes, even though one leafy genotype (P14) facilitated above average mixture yields, based on our results, this strategy can as well not be recommended without reservation, due to the high GxE that was observed with this plant type (see section 4.4.3.1, p.72) and its strong competition towards barley. Thus, strategy c), increasing stipule area, appears as the most viable strategy to convey medium sized, semi-leafless plants with good competitive ability for light at the top layer of the canopy. We observed a likely positive correlation between stipule length and GMA. If this is confirmed, the high heritability and broad phenotypic variance of this trait would make it an attractive target for selection for increased GMA in general and for marker assisted selection in particular. Our observation on stipule size is in line with other studies (Jacob *et al.*, 2016). Whatever the selection method, phenotypically or by marker assisted selection – phenotyping should be done in mixed stand since the trait showed a certain plasticity in mixed compared to pure condition, with certain genotypes not maintaining a high leaf size in mixture (data not shown but see Fig. 19 as an example). Biomass was a proxy for plant length and its use as a selection criterion is thus to be gauged in the context of lodging (see following paragraph). Same as for common bean in (mixed crop with corn, Zimmermann *et al.*, 1984), also in pea traits could be identified that were not correlated with yield in pure stand but with yield when pea was combined with barley (onset of pea flowering and stipule length) and thus deserve a special focus in dedicated breeding programs of pea for this specific mixed crop. While a linear regression was applied to the effect-trait relationships, non-linear functions might yield optima for key-trait values, such as early vigor measurements, to maximize GMA, Pr- or As-effects.

The heritability of total mixture yield was moderate ( $H^2 = 0.56$ ) and some superior genotypes, all from the semi-leafless type, showed potential to produce above average mixture yields and might give important insights on how to improve mixture yields by targeted selection. The fact that this was achieved solely by genotypes of medium stature (P12, P20 and P25, all mapping to sector V, i.e., having both positive Pr and As effects) indicates that this group of genotypes explored free niches in the plant community possibly more efficiently than long genotypes and – with great confidence – more efficiently than short genotypes. Interestingly this would be in contradiction to the positive correlation between Pr effects and GMA, which suggests that highest GMA can be found not among medium sized genotypes but among genotypes with high Pr effects, and thus mostly with long stature. This conclusion must be drawn with care, since the genotypes out of the ‘long’ group with the highest Pr effects (P02, P06 and GM-long) all were at the verge of strong lodging. Therefore, trying to increase GMA by long plants as suggested by Annicchiarico *et al.* (2019) is only possible to a certain degree, ultimately limited by lodging and, as a consequence, harvest losses of very long genotypes (if not compensated by increased resistance to lodging in the cereal partner). Our observation also underpins the theory that productivity of plant communities is governed by finding an optimal balance between competitiveness of the two species (Wendling *et al.*, 2017), avoiding too strong selection effects of any of the species involved (Brooker *et al.*, 2021; Schöb *et al.*, 2015).

## 5. Chapter 3 – Key-trait-based and pure-stand-based indirect selection for mixture yield of pea

### 5.1. Introduction

Selection for mixture yield of one species in a mixed crop can be done directly, by selecting for the target variable (mixture yield) or indirectly, by selecting for traits or other auxiliary measurements that are genetically correlated with the target variable (see e.g. (Le Campion *et al.*, 2014 for an application). Direct selection for mixture yield is costly since plot trials with several replicates must be sown and harvested over multi-environment trials. This is not feasible for all stages of a breeding program, especially not in early stages where the number of genotypes to test is considerable. Indirect selection methods are thus a welcome method to mitigate this problem. Two potential strategies for indirect selection could be using trait measurements of known key-traits for mixture performance in small plots or single row configurations and combining them into a selection index or – if such an index cannot be created – indirect selection for mixture yield using pure stand yield data. While direct selection and selection by pure stand yield represent ‘trait-blind’ approaches, the key-trait selection index represents a ‘trait-informed’ approach, which can also serve to ‘design’ mixtures with particular features or ‘baskets of services’ (Barot *et al.*, 2017). If selection via pure stand yield data is used, selection based on this criterion could only be carried out at later stages of a breeding program where enough seed is available for doing multi-environment plot-trials. Indirect selection via key trait selection is a cost-effective way to screen for suitable genotypes already in early stages of a breeding program where yield data is not available due to the low availability of seeds and the high number of potential test candidates. Key traits should thus be (a) easy and quick to screen at small plots or single rows, (b) of low cost in measurement (c) highly correlated to the mixed crop trait response (e.g. total mixture yield, nutritional traits etc.), and (d) highly heritable. The indirect selection methods can be compared to the direct selection and depending on the efficiency of the indirect selection vs. the direct selection, breeding schemes can be shaped accordingly (early performance testing of many genotypes if indirect selection is ineffective or indirect selection via trait observations in the other case). A validation experiment was performed in which the mixture yields of 17 pea genotypes were predicted by either direct selection (GMA) or indirect selection via pure stand yield data or a key-trait selection index based on pure stand trait data. Furthermore, GMA can only be used as a proxy for direct selection when SMA does not play a considerable role and does not impair the predictability of mixture performances due to GMA, which was the case in our findings (see chapter 2), but which is based on a set of barley genotypes that might not represent the wholeness of agronomically relevant barley genotypes and SMA might well play a role in other contexts, depending on the genotypes used. However, the results of this comparison of different selection approaches are an important first step that will help to design dedicated breeding schemes for spring-pea in mixed cropping with spring-barley.

## 5.2. Materials and methods

For a description of the plant material, site, soil and management data, please refer to the section 'Experimental setup and methodology applied', subsection 'Experiment 2 (2020)', page 37ff.

### 5.2.1. Traits used in the analysis

GMA of the pea genotypes was calculated based on experiment 1 after excluding four multi-cultivar mixtures (unsuitable due to heterogeneity of trait measurements), three genotypes with poor germination in 2018 (P16, P17, P30), two genotypes with insufficient maturity (P15, P23), and a genotype with extreme lodging (P28). In total 22 genotypes were used for the estimation of GMA. Trait data of these genotypes was extracted from the raw data of 2018 and 2019 and averaged by genotype. See Table 7 for an overview on the measured traits. These putative key traits were measured in pure stand condition in experiment 1. The stipule parameters were measured on all genotypes only in the year 2018 of experiment 1, whereas they were measured only on a subset of genotypes in the year 2019 of experiment 1. Due to the high heritability ( $H^2$  0.87) of stipule parameters the one-year data of 2018 was treated as equivalent to the two-year data of the other measurements. Stipule area is a derived parameter from stipule length and width, using the ellipsis formula.

**Table 7.** Traits measured in the 2018 and 2019 trials in pure stands. Stipule data was assessed at all genotypes in 2018 and at a subset in 2019.

<b>Trait</b>	<b>Measuring unit</b>	<b>Measuring time-point (BBCH pea)</b>
Early vigor	1-9 scale	25-36
Early canopy height	cm	19
Early canopy height at onset of flowering	cm	34-39
Canopy height at grain filling	cm	74-79
Canopy height at maturity	cm	85
Onset of flowering	days after 1st of January	65
Total biomass	1-9 scale	65
Stipule length	mm	65
Stipule width	mm	65
Stipule area	mm <sup>2</sup>	65
Lodging at grain filling	1-9 scale	74-79
Lodging at maturity	1-9 scale	85
Grain yield	t/ha	85

### 5.2.2. Selection for non-redundant trait-measurements

In a linear model with GMA as dependent variable and all 13 traits as predictor variables some traits exhibited high multicollinearity, expressed by a variance inflation factor (VIF) >10. In order to assess multicollinearity, each trait was checked for its correlation with the other traits. If a trait had a correlation coefficient > 0.6 with one or several other traits, among these multicollinear traits the one with the highest correlation with GMA was selected for further analysis and the others were discarded. The following four key-traits were used for further analysis: early vigor, onset of flowering, biomass, and stipule length. After using only these traits, the VIFs decreased dramatically in a linear model where again GMA was the dependent variable. VIFs of these traits ranged from 1.5 to 2.4 and averaged 2.0, and thus are no cause for concern (Field *et al.*, 2012).

### 5.2.3. Assessing predictive power of traits via cross-validation

As a measure on how high the predictive power of each trait on GMA was, for each trait separately, a cross-validation with three folds, i.e., 2/3 of arbitrary genotypes being in the training set and the remaining 1/3 in the validation set was performed 100 times with arbitrarily drawn genotypes for the two sets at every run. Cross validation was done using the GNU R package caret (Kuhn, 2008) with GMA as dependent variable and the corresponding trait as predictor. For each trait, one hundred  $R^2$ s between the predicted GMA of the validation set (as calculated by multiplying the fitted coefficients of the genotypes in the training set with the corresponding trait values of the genotypes of validation set) and the actual GMA values of the validation set were computed. The average of these one hundred  $R^2$ s (and their broad sense heritability  $H^2$  in brackets) were 0.25 (0.56) for early vigor, 0.17 (0.87) for onset of flowering, 0.20 (0.81) for biomass, and 0.28 (0.87) for stipule length, respectively.

### 5.2.4. Creation of the key-trait selection index

An individual weighing factor was created for each key-trait, multiplying this average cross validated  $R^2$  (between trait-predicted GMA and true GMA, see above) with its  $H^2$ . Trait values were z-transformed. In the case of onset of flowering the correlation of a trait with GMA was negative and therefore z-transformed trait-values were multiplied with -1, in order to have all z-transformed traits positively correlated with GMA. For each trait, the z-transformed values were multiplied with the trait's weighting factor and these products summed up across all four key-traits for each genotype to form an additive key-trait selection index. The final additive key-trait selection index showed an  $R^2$  of 0.36 with the 2018/2019 GMA values.

### 5.2.5. Selection of genotypes according to their GMA-, pure-stand- or key-trait-index-performance

For each selection method, GMA, pure stand, or key-trait-index, a positive and a negative selection was done, using the best four and worst four genotypes according to the selection method. The remaining experimental space allowed to choose a fifth genotype for the positive selection with the key-trait-index (P05 – Navarro). Therefore, from the initial 22 genotypes 17 entries were chosen by

positive or negative selection with the different selection methods used, with seven genotypes being chosen by more than one selection method. Selected genotypes and the selection method they were chosen with are shown in Table 8.

**Table 8.** The seventeen genotypes used in the experiment with their predictors for mixture yield: GMA- and pure stand performance in 2018 and 2019 and key-trait selection index based on pure-stand trait-data of 2018 and 2019. With each predictor a positive (the four to five best performing genotypes) and a negative selection (the four worst performing genotypes) was done. Positive selection is indicated by numbers '1-4' ('1-5' in the case of key-trait selection) and negative selection with numbers from '7-10', with '1' and '10' being the best and worst performing genotype according to the predictor, respectively.

Pea code	Pea cultivar	GMA estimate 2018/19 [t/ha]	Pure stand yield 2018/19 [t/ha]	Key-trait selection index	Selection GMA	Selection pure-stand yield	Selection key-trait-index
P01	SG-L 7647	-0.24	3.71	-0.89	10		10
P02	Impuls	0.17	4.16	0.68			1
P03	Astronaute	0.03	4.37	-0.02		3	
P05	Navarro	0.01	3.90	0.27			5
P07	Angelus	-0.15	3.70	-0.5	9		7
P08	Salamanca	0.23	4.44	0.19		2	
P11	Karpate	0.11	3.86	0.41			3
P12	Kayanne	0.32	4.04	0.08	3		
P13	Mytic	-0.11	3.87	-0.68			9
P14	Protecta	0.40	3.19	0.22	2	9	
P19	Bockros	0.10	3.69	0.49			2
P20	Volt	0.43	4.19	0.34	1		4
P24	Peps	0.08	4.35	0.18		4	
P25	Karioka	0.30	4.57	-0.04	4	1	
P27	Milwa	-0.12	3.28	-0.57		8	8
P29	Natura	-0.15	2.90	-0.28	7	10	
P31	Starter	-0.15	3.66	0.02	8	7	

## 5.2.6. Statistical analysis

An ANOVA and estimated marginal means of pea cultivars were calculated using a fixed effects model of the form:

$$y_{ijlm} = \mu + l_l + r_m(l_l) + G_{p_i} + G_{b_j} + S_{ij} + G_{p_i}l_l + G_{b_j}l_l + S_{ij}l_l + E_{ijlm} \quad (15)$$

with  $y_{ijlm}$  the total mixture yield of the  $i$ -th pea cultivar mixed with the  $j$ -th barley cultivar in the  $l$ -th location and the  $m$ -th block of experiment 2,  $\mu$  the intercept of mixture yields,  $l$  the effect of the  $l$ -th location,  $r$  the effect of the  $m$ -th block (replication) nested in location,  $G_{p_i}$  and  $G_{b_j}$  the GMA effects of the  $i$ -th pea cultivar and the  $j$ -th barley cultivar, respectively,  $S_{ij}$  the SMA effect, i.e. interaction, of the  $i$ -th pea cultivar with the  $j$ -th barley cultivar and  $E_{ijlm}$  the residual of total mixture yield,



contributed by the m-th replication of the i-th pea genotype combined with the j-th barley genotype in the l-th location. The three-way interaction of SMA (GMA x GMA) x location was left out for means of simplicity. Based on this model, estimated marginal means (EMMEANS; Lenth, 2019) were calculated for pea cultivars both within locations and across locations (which equals a GMA estimation of experiment 2). The GMA estimates across both locations were used for the correlation analysis and the comparison of the efficiency of direct versus indirect selection.

### 5.2.7. Efficiency of indirect selection

The efficiency of indirect selection was determined by dividing the coefficient of correlation of the key-trait selection  $\rho_i$  (correlation of key-trait index derived from trait measurements in pure stand in the 2018 and 2019 trials to predict 2020 mixture yield, see section 5.2.4) with the coefficient of correlation from the direct selection  $\rho_d$  (GMA derived from 2018 and 2019 trials to predict 2020 mixture yield).

$$\frac{\rho_i}{\rho_d} \quad (16)$$

Efficiency of selection based on pure stand yield was assessed in the same manner, then using the coefficient of correlation of pure stand selection  $\rho_p$  (correlation pure stand yields, derived from measurements in the 2018 and 2019 trials to predict 2020 mixture yield) in the numerator.

## 5.3. Results

### 5.3.1. Repeatability and analysis of variance

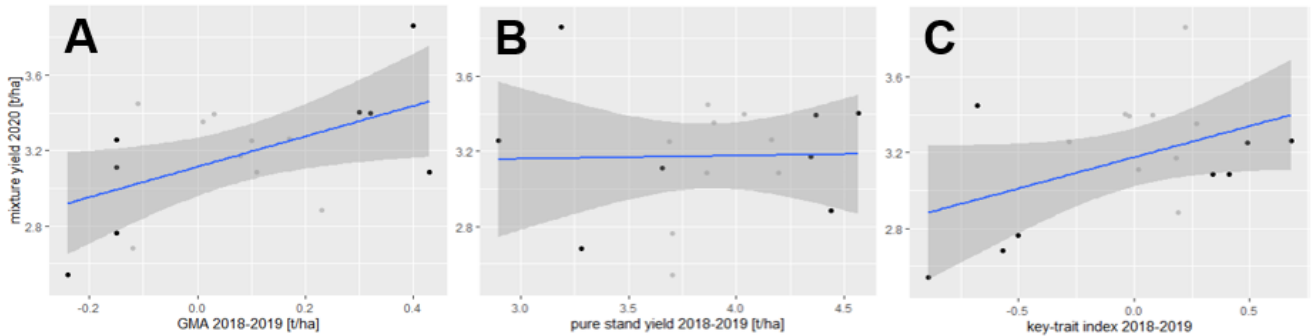
Repeatability of yields in the 2020 trials were low within locations and across locations. Within Fislisbach, the  $R^2$  of total yield between replications ranged from 0.07 to 0.27 and within Uster from 0.07 to 0.30 (correlations of each specific pea-barley mixture between the repetitions). GMA estimates of pea for each location (Fislisbach and Uster) were correlated with an  $R^2$  of 0.46. Pea and barley cultivars had a highly significant effect on total yield (table 9, following page).

**Table 9.** Anova table of 2020 data with total mixture yield as dependent variable.

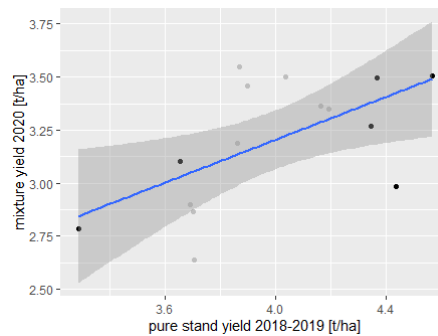
	Df	Sum Sq	Mean Sq.	F-value	p-value
location	1	0.379	0.3787	1.4489	0.2309
pea	16	17.996	1.1247	4.3035	<0.0001 ***
barley	1	4.078	4.0776	15.602	0.0001 ***
pea:barley	12	4.491	0.3743	1.432	0.1591
location:pea	16	5.432	0.3395	1.299	0.2069
location:barley	1	0.65	0.6499	2.4866	0.1172
Residuals	132	34.498	0.2614		

### 5.3.2. Mixture yield prediction with different methods: GMA, pure stand yields and key-trait index

The correlation coefficient of GMA of experiment 1 vs. GMA of experiment 2 (direct selection) was  $\rho_d = 0.57$  ( $p=0.0169$ , Fig. 16A). The mean of the genotypes from positive selection was with 3.59 t/ha significantly higher compared with genotypes from negative selection 2.99 t/ha ( $p=0.0147$ ). The correlation coefficient of pure stand yields of experiment 1 vs. GMA of experiment 2 (indirect selection with pure stand yields) was  $\rho_p = 0.07$  ( $p=0.7754$ ) when leafy genotypes P14 and P29 were included (Fig. 16B). The means of the genotypes from positive (3.31 t/ha) and negative (3.31 t/ha) selection did not differ significantly in this case ( $p=0.4974$ ). When the leafy genotypes P14 and P29 were removed, the correlation coefficient (indirect selection with semi-leafless pure stand yields) was  $\rho_p = 0.60$  ( $p = 0.0182$ ) and the means of the positive (3.31 t/ha) and negative selection (2.94 t/ha) had a tendency to differ significantly ( $p=0.0946$ ; Fig. 17). The correlation coefficient of indirect selection via the key-trait index based on trait data from experiment 1 and GMA of experiment 2 was  $\rho_i = 0.36$  ( $p = 0.1608$ ). The mean difference of the genotypes from positive (3.19 t/ha) and negative (2.96 t/ha) selection was not significant ( $p=0.1731$ ).



**Fig. 16.** Correlation of prediction (selection method, x-axis) and observation (mixture yield in 2020, y-axis) of 17 spring pea cultivars. Dark dots indicate the genotypes from negative (left) and positive (right) selection of each method. (A) GMA 2018-2019 vs. mixture yield 2020 (B) pure stand yields 2018-2019 vs. mixture yield 2020 (C) key-trait index 2018-2019 vs. mixture yield 2020.



**Fig. 17.** Correlation of prediction (pure stand yield 2018-2019, x-axis) and observation (mixture yield in 2020, y-axis) of 15 spring pea cultivars, omitting leafy genotypes P14 and P29 (see Fig. 16 B for comparison). Dark dots indicate the genotypes from negative (left) and positive (right) selection of each method.

### 5.3.3. Efficiency of indirect selection

The efficiency of indirect selection for mixture yield, using the key-trait index that consists of early vigor, biomass, onset of flowering and stipule size was  $\frac{\rho_i}{\rho_d} = \frac{0.36}{0.57} = 0.63$ . Mixture yield could be predicted with key-trait data with an efficiency of 63%. The efficiency of indirect selection using pure stands was not computed due to the exceptionally low correlation coefficient of pure stand selection  $\rho_p$  of 0.07. However, if the leafy types were left out, the following efficiency was calculated  $\frac{\rho_p}{\rho_d} = \frac{0.60}{0.57} = 1.05$ . Pure stand yields of semi-leafless peas predicted mixture yield with an efficiency of 105%.

## 5.4. Discussion

Even though certain replications were badly correlated with others within locations, good repeatability of the pea genotypes' genotypic effect on mixture yields (GMA) over locations was given with an  $R^2$  of 0.46. This is further underlined by the significant genotypic effects of pea over both locations (table 9). Direct selection using known GMA-data of genotypes significantly correlated with mixture yield and can serve as a selection criterion for peas in pea-barley mixed crops. This result does not come to a surprise, given, the  $H^2$  of total mixture yield of 0.59 (see chapter 2). Indirect selection using pure-stand data was completely uncorrelated with mixture yields in 2020. This is unexpected, since in cases in where different species combinations were used, genotypes showed a

(genetic) correlation coefficient of between 0.25 and 0.83 (Annicchiarico *et al.*, 2019). These findings underline the species-specificity of the usefulness of pure stand yield data as a predictor for mixture yield and it cannot be ruled out as a predictor for mixture yield in any mixed crop *per se*. However, to conclude that in our case the correlation was nil and thus our results suggest that pea pure stand data is useless to predict mixture yields in pea-barley mixed crops, is wrong. Pure stand data contained two leafy genotypes, P14 and P29. These genotypes are represented as the two leftmost dots in Fig. 16 B. Doing a separated analysis omitting these genotypes – in analogy to the analysis shown in Fig. 10 A (p. 63) – would have resulted in a dramatic increase in efficiency of indirect selection to 105% and using pure stand yield data as an indirect selection method is thus highly dependent on this trait. Pure stand yield-data is often readily available since most pea-breeding programs are dedicated to pure stand cultivation and the suitability of genotypes for mixed-cropping would be an added value to a cultivar. Indirect selection using key-trait index showed a medium correlation with 2020 yields of 0.36 and a selection efficiency of 63% compared with direct selection. This indicates that the use of trait-based indirect selection shows potential to be used as a selection approach in early phases of breeding programs for pea (for mixed cropping with barley), where seeds are too scarce to do plot trials, however phenotypic observation of these predictor traits for later performance in single rows or small nursery-plots is achievable, cost effective and quick. Likewise, if pure-stand yield information is available, it can be used due to its high efficiency as an indirect selection criterion. The results confirm the observations made in chapter 2: pure stand data, as well as trait data can serve as good predictors for mixture yield, pure stand data however only, if leafy genotypes are not considered. The three-step approach used to create the key-trait index – 1) selection for non-redundant trait measurements, 2) assessing predictive power of traits via cross-validation and 3) creation of the index using heritability as a weight – proved as a useful algorithm to create this index. However, since no other approach (e.g., a multiple regression, using all 13 traits, or no weighting by heritability) was contrasted, no comparisons can be made regarding the chosen approach. Furthermore, a cross validation, using a common set of mixtures that was grown in all three experimental years, could have provided even more solid results on the three selection approaches. A limitation of the study might be the measurement of trait data in pure stand. Certain traits (stipule parameters e.g.) have shown to display a certain plasticity between pure and mixed condition (data not shown). Thus, in further studies, it should be investigated, if a key-trait index, based on measurements made in mixed stand (and consequently also doing indirect selection in mixed condition) yields similar or potentially higher power to predict GMA. However, our results give a first indication on pathways to pursue in designing dedicated breeding schemes for pea in mixed cropping with barley. Based on these findings, I recommend using a key-trait-index (measured in pure stand) in early phases of a mixed cropping breeding program for spring-peas. In later steps, if only semi-leafless genotypes are the target material, indirect selection via pure stand yield-data should be pursued, and if leafy genotypes are the target material, direct selection in mixed stand should be done.

## 6. General conclusions

The fact that no specific mixing ability (SMA) was detected suggests that currently available pea and barley material can readily be combined based on prior knowledge of their GMA. This circumstance facilitates future efforts of selecting superior genotypes for this mixed crop as well as subsequent adoption by the seed-value chain and farmers. Even though being absent in our results, SMA should be regularly monitored in future breeding efforts, in particular in the case a larger range of barley genotypes would be considered. Incomplete factorial designs have proven especially useful in this context. They showed the capacity to estimate variance components of GMA/SMA as well as individual genotypic effects in a resource efficient way. Their usefulness has been proven in theory (simulation study) and their feasibility was demonstrated empirically (Experiment 1). Along with the mixture yields, pure stand yields were measured and can serve as a fair predictor for the total mixture yield to be expected and of pea fraction yield in the mixture (for semi-leafless pea cultivars). The usefulness of pure stand yields as a predictor for mixture yields of (semi-leafless) peas was confirmed additionally by experiment 2. My observation of a medium correlation between pure stand and mixture yields neither justifies in my eyes a completely independent breeding program for mixed cropping, nor will a selection by pure stand yields alone lead to high selection gain in mixed cropping. Rather should key-traits be integrated as an additional selection tool in early generations of a breeding program to separate useful material for mixed cropping at these stages and to test it in mixture (and parallel for pure stand performance) in later stage. As for traits, my results display the importance of early vigor for improving GMA in pea. Onset of flowering of pea was significantly negatively associated with its As effect on barley yield, while not being associated with its Pr effect. This might be evidence for a more complementary resource use pattern, potentially for light, between pea and barley with earlier flowering pea genotypes. For stipule size, a likely positive correlation with GMA was observed. If this is confirmed, the high heritability and broad phenotypic variance of this trait would make it an attractive target for selection for increased GMA in general and for marker-assisted selection in particular, Genotypes of medium stature explored free niches with great confidence more efficiently than short genotypes. A key-trait selection index that included data on early-vigor, onset of flowering, stipule length and biomass yielded a sufficiently high efficiency for indirect selection of 64%. Leafy types bear both potential, and risk concerning future breeding activities and based on my results, I would not recommend this plant type neither to breeders, nor to farmers, as long as the reasons for the low yield-stability of this plant type are not known. If this issue can be mitigated somehow, they would represent an interesting plant type however, as they have a strong early vigor and competitive ability against barley. For other potential key-traits (e.g. I did not investigate root morphology in my thesis) determining the biological interaction function (BIF) of traits can serve to identify traits that help occupying the free niche space in mixtures by selecting for traits that cause commensalistic – or a least net-commensalistic – behavior, i.e., a positive effect on GMA even though their effect on Pr and As effects is to a certain degree antagonistic. Concluding on traits, I suggest a pea ideotype for mixed cropping with barley that has strong early vigor, is regarding its plant height in an optimum between a medium- and

long-sized plant while having sufficient lodging resistance, has long stipules (or large stipule area, respectively), and which is of early flowering.

The use of more diverse genetic material (i.e. genotype mixtures or multi-line mixtures), especially in the pea mixture-component, has shown potential to stabilize pea fraction yields and the mixture ratio across environments. I suspect that the higher stability gained by increasing the diversity at the intra-species level is governed by the same stabilizing mechanisms that are at play by increasing diversity at the inter-species level (mixed cropping), i.e. by increased niche complementarity and adaptability (Meilhac *et al.*, 2019). The fact that a GM (GM-long), containing genotypes with a broad range of three important traits for mixing ability – plant length, leafiness and lodging resistance – outperformed its components (i.e. its GMA was significantly higher than the GMA expected by the average of all of its components), might be caused by a positive effect of trait-divergence of one or several of these three traits. This might sound counterintuitive. Why would it be beneficial to include strongly lodging plants in such a mixture? But plant length and lodging resistance is to a certain degree (negatively) correlated, thus, less lodging (short) plants support the potentially higher yield capacity of longer genotypes (that are otherwise prone to lodging as homogeneous pea cultivar) and pea as a whole profit from the improved competition against barley provided by the ‘collaboration’ of these two plant types. However, this should be explored by further research, as the trait-divergent GM only was created accidentally in our case and all other GMs were uniform for one of these important traits (plant length and leafiness). This should be done by doing more systematic combinations of GMs that diverge for one or several of the beforementioned traits. This systematic use of intra-specific diversity could unlock the next level of mixed crop performance and stability.

To date I could not find a coherent explanation for the observation, that stability in Pr effects, but not in As effects is related to stability in GMA and it was not intuitively understandable to me why only the stabilizing effect on the own fraction yield but not on the companion crop played a role in stabilizing the whole mixture yield. This should be a matter of further research as stability of mixture yields is of equal importance as a high overall yield.

The fact that gains in GMA by higher Pr effects are realized without a tradeoff by a negative relationship between As effects and GMA point towards the commensalistic situation in mixed stands, i.e. that besides competition for shared resources (especially soil N at early growth stages and light at later stages) free resource-niches for the two plants exist. It indicates that there’s yet unlockable potential in both species to select genotypes that occupy free niche space in mixture even more effectively and thus realize higher mixture. Genotypes that significantly positively deviated from the tradeoff between Pr and As effects can be seen as evidence for this observation.

Breeding efforts on the vast majority of crops today focus on pure-stand yield performance. Maybe this situation will change with increasing popularity of mixed cropping in the near future, but if so, this will only happen progressively. It is thus unlikely that breeding efforts of complete breeding programs will focus mainly on mixed cropping. (However: in Switzerland two small, recently started dedicated breeding programs for mixed cropping exist – one for field pea in combination with barley of Getreidezüchtung Peter Kunz and another one for common bean in combination with corn of

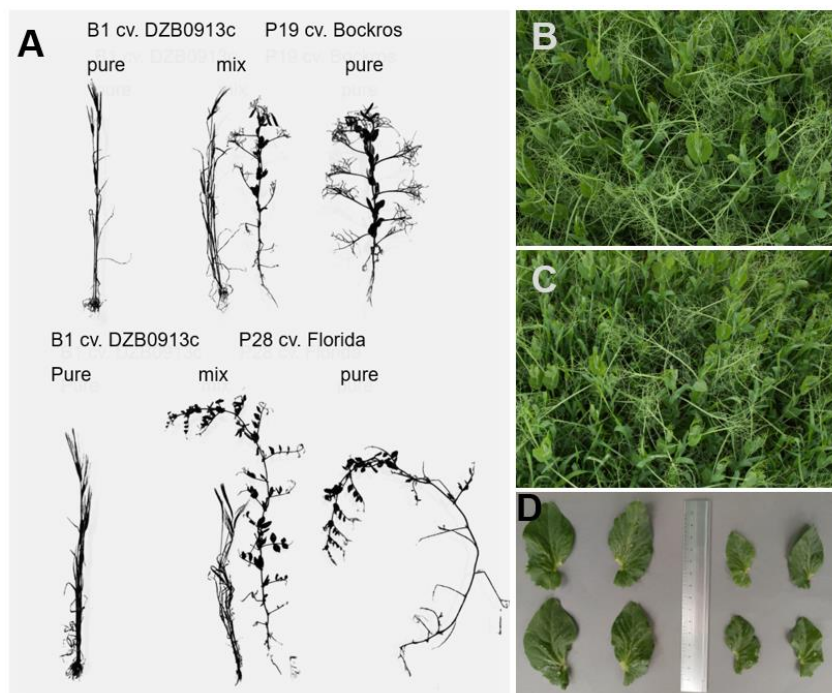
Sativa Rheinau AG). It is more likely that breeding will focus on 'dual-use' genotypes that perform well in both ways of usage and that indirect selection by pure-stand yields will be used or that breeding strategies will split testing environments for pure- and mixed-stand cultivation at relatively late stages of the breeding scheme, since pure-stand cultivation will still be the main target environment for the nearer future. Therefore, the question to what extent pure-stand yield performance is correlated with mixed-stand yield performance is one of imminent importance. Based on the extent of the predictive capacity of pure stand yields, either no dedicated breeding programs (high correlation) or completely independent dedicated breeding programs have to be set up (low correlation) or something in between (medium correlation). Even in case where no dedicated program is necessary, it might be useful to develop experimental designs and statistical approaches to assess the candidate varieties for their ability to grow well in mixtures.

## 7. Supplement: visual impressions of the observed phenomena

Finally, for the brave reader that has made it until here, a few visual impressions are given as a reward. These comprise different observations made during the field seasons of 2018 to 2020.



**Fig. 18.** Images of a strongly lodging genotype and a genotype with good lodging resistance in pure stand.

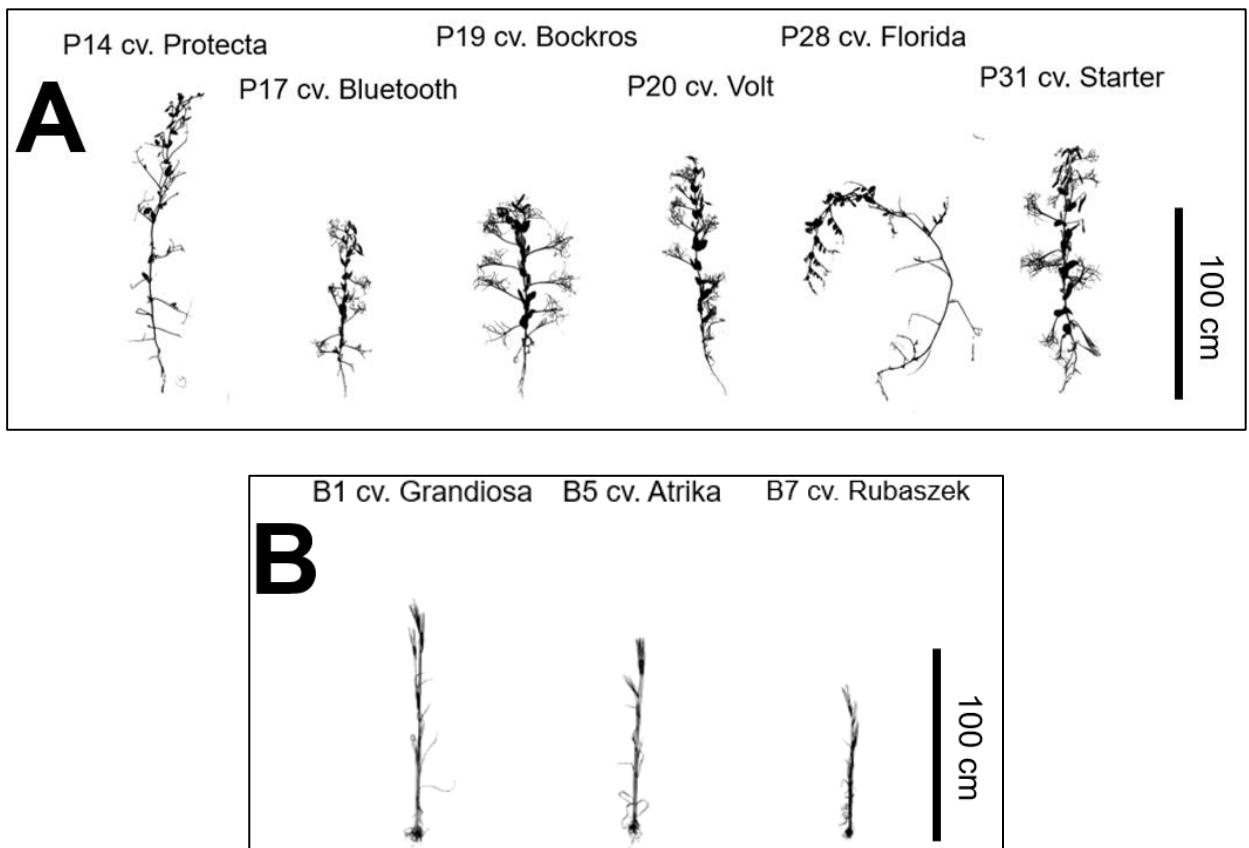


**Fig. 19.** Phenotypic reaction of pea on mixed condition with barley. Peas generally showed reduced growth in mixture with barley which became evident in lower stature and shorter tendrils (A, top row), and reduced stipule leaf size in mixture (C and D, right) as compared to pure stand (B and D, left). Barley generally did not show this reaction and only showed reduced growth when combined with extremely vigorous plants, such as P28 (A, bottom row).





**Fig. 20.** Measurement of stipule length.



**Fig. 21.** Images of six exemplary pea (A) and three exemplary barley genotypes (B) displaying the morphological diversity in the genotypes used in the experiments. Pictures were taken in Fislisbach 2019.

## 8. Appendix

### 8.1. Table. Error correlation

**Table A1.** Comparison of two different analysis-approaches of fraction-yields of a mixed cropping experiment (experimental design D 8x30i, see Fig. 4), tested with 1000 simulated datasets. SMA was assumed to be 0 and correlation of errors of pea and barley fraction yield in a plot was assumed to be either -0.9, -0.5 or 0. The first approach estimates the parameters with a univariate (formulas (5) and (6)), the second with a bivariate model (formula (7)).

Parameter	Truth	Model	Error correlation			
			-0.9	-0.5	0	
pea Pr variance	6.1	univariate	mean	6.194	6.177	6.205
			variance	5.572	5.516	5.611
		bivariate	mean	6.195	6.174	6.208
			variance	5.582	5.413	5.490
pea As variance	7.46	univariate	mean	7.509	7.498	7.481
			variance	6.050	6.151	6.331
		bivariate	mean	7.507	7.503	7.478
			variance	5.981	6.197	6.333
pea Pr-As covariance	-4.11	univariate <sup>1</sup>	mean	-3.544	-3.074	-2.481
			variance	2.875	2.408	1.948
		bivariate	mean	-4.176	-4.188	-4.139
			variance	4.175	3.843	3.597
barley Pr variance	6.06	univariate	mean	5.909	5.918	5.945
			variance	13.064	12.995	12.638
		bivariate	mean	5.968	5.987	5.998
			variance	12.820	13.025	12.741
barley As variance	1.54	univariate	mean	1.553	1.564	1.546
			variance	1.724	1.709	1.737
		bivariate	mean	1.552	1.556	1.537
			variance	1.726	1.690	1.702
barley Pr-As covariance	-2.69	univariate <sup>1</sup>	mean	-2.122	-1.964	-1.730
			variance	3.247	2.889	2.522
		bivariate	mean	-2.669	-2.674	-2.662
			variance	3.972	3.724	3.501
Error variances of pea	8.43	univariate	mean	8.403	8.401	8.414
			variance	1.717	1.706	1.718
		bivariate	mean	8.406	8.411	8.429
			variance	1.712	1.720	1.730
Error variances of barley	6.09	univariate	mean	6.099	6.129	6.147
			variance	0.901	0.882	0.855
		bivariate	mean	6.090	6.122	6.147
			variance	0.875	0.875	0.860
Error covariances in the bivariate model	-3.58/ -6.45/0.0	bivariate	mean	-6.436	-3.560	-0.019
			variance	1.119	0.774	0.612
pea Pr truth vs. BLUP correlation	1	univariate	mean	0.843	0.843 <sup>a</sup>	0.844
			variance	0.004	0.004	0.004
		bivariate	mean	0.834	0.838 <sup>a</sup>	0.849
			variance	0.007	0.006	0.007
pea As truth vs. BLUP correlation	1	univariate	mean	0.894	0.895 <sup>a</sup>	0.895
			variance	0.002	0.002	0.002
		bivariate	mean	0.900	0.892 <sup>a</sup>	0.897
			variance	0.003	0.002	0.002
barley Pr truth vs. BLUP Correlation	1	univariate	mean	0.936	0.936 <sup>a</sup>	0.937
			variance	0.004	0.004	0.003
		bivariate	mean	0.938	0.927 <sup>a</sup>	0.928
			variance	0.009	0.008	0.009
barley As truth vs. BLUP Correlation	1	univariate	mean	0.788	0.787 <sup>a</sup>	0.789
			variance	0.024	0.025	0.024
		bivariate	mean	0.725	0.739 <sup>a</sup>	0.756
			variance	0.075	0.070	0.063

<sup>1</sup>to be read with care: these covariances are estimated "reversely" by the correlation and standard deviation of the producer and associate BLUPs

## 8.2. Table. Comparison univariate versus bivariate approach

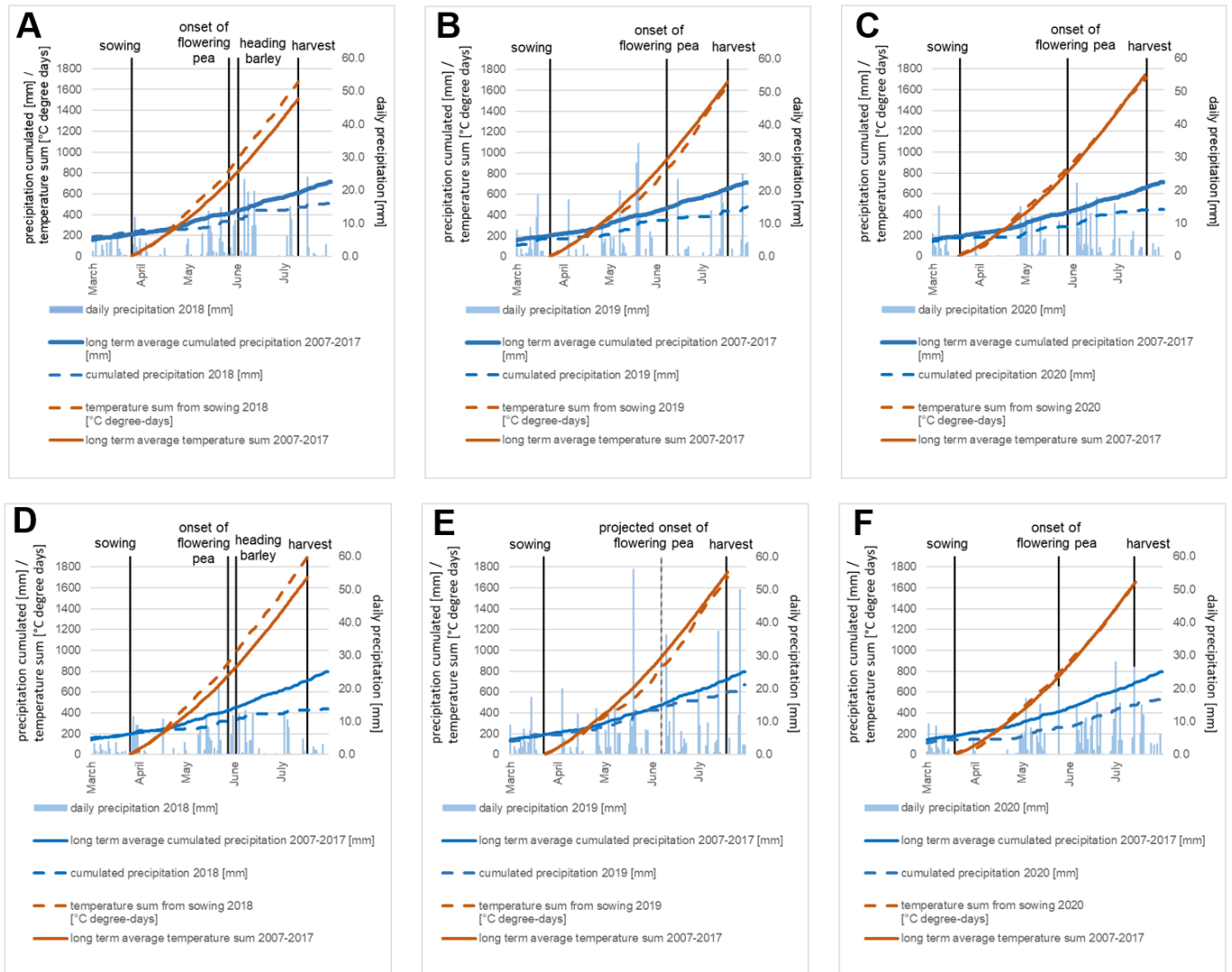
**Table A2.** Confidence intervals (CIs) and truth vs. BLUP correlation of the “error correlation -0.5 scenario” (Table A1). The uni- and bivariate models are described in formulas (5), (6) and (7). Since producer and associate effects of a genotype sum up to its GMA, for completeness also the GMA variance is given in brackets. The ± sign indicates the 95% confidence intervals for the variance estimates.

	Parameters	Model	Truth	Estimate and 95% CI			Mean of correlations BLUPs vs. true effects <sup>1</sup>
GMA pea (5.13)	pea Pr variance	univariate	6.10	6.18	±	0.15	0.84 <sup>a</sup>
		bivariate		6.17	±	0.14	0.84 <sup>a</sup>
	pea As variance	univariate	7.46	7.50	±	0.15	0.90 <sup>a</sup>
		bivariate		7.50	±	0.15	0.89 <sup>a</sup>
	pea Pr-As covariance	bivariate	-4.11	-4.19	±	0.12	-
GMA barley (2.11)	barley Pr variance	univariate	6.06	5.92	±	0.22	0.94 <sup>a</sup>
		bivariate		5.99	±	0.22	0.93 <sup>a</sup>
	barley As variance	univariate	1.54	1.56	±	0.08	0.79 <sup>a</sup>
		bivariate		1.56	±	0.08	0.74 <sup>a</sup>
	barley Pr-As covariance	bivariate	-2.69	-2.67	±	0.12	-

<sup>1</sup> means of rho-z-rho transformed Pearson correlations, see materials & methods, letters indicate significant differences between uni- and bivariate model, with p<0.05 (no significant differences for any parameter)

### 8.3. Figures. Climate

Climate data and key events of the four experiments have been summarized graphically (see Fig. A3). The Fislisbach data is the average value of the nearby Swiss federal measuring stations Lupfig and Künten, the Uster data is the average of the nearby stations Seegräben, Stäfa and Lindau.



**Fig. A3.** Climate and key events in the trials 2018-2019. The top row (A, B, C) shows the location Fislisbach, the bottom row (D, E, F) location Uster. In the columns, the two experimental years are shown with 2018 in the left column (A, D), 2019 in the second (B, E), and 2020 (C, F), respectively. Names of months demark the beginning of a month on the x-axis. The solid orange lines show the expected temperature sum in °C-degree-days between the sowing and harvesting date based on long term average daily temperatures (2007-2017) at each location whereas the dashed orange line shows the actual temperature sum within this time period in an experiment. In analogy, the solid blue line is showing the long-term average cumulated precipitation from January 1<sup>st</sup> onwards and the dashed line shows the actual cumulated precipitation from January 1<sup>st</sup> onwards of the given year and location of an experiment. Temperature sums and cumulated precipitation measurements are scaled on the left y-axis while daily precipitation events, shown as light blue columns, are scaled on the right y-axis.

## 8.4. Table. Soil parameters

**Table A4.** Soil parameters of the four different experiments.  $N_{min}$  comprises the total amount of water soluble  $NO_2^-$ ,  $NO_3^-$  and  $NH_4^+$ . All measures refer to the 0-90 cm soil layer.

Location	Year	$N_{min}$ [kg/ha]	Mn [mg/kg]	Soil components [%]				Soil type
				humus	clay	silt	sand	
Fislisbach	2018	41.9	294.0	2.4	14.1	31.3	52.2	sandy loam, brown earth
	2019	35.0	241.0	2.7	14.6	44.5	38.2	loam, brown earth
	2020	76.6	233.0	2.5	23	24.8	49.7	sandy clay loam, brown earth
Uster	2018	93.2	133.0	4.3	25.1	28.9	41.7	loam, gley
	2019	117.8	87.0	4.2	35.9	26.6	33.3	clay loam, gley
	2020	54.9	174.0	2.5	21.3	28.2	48	loam, gley

## 8.5. Figure. Experimental design

	P00	P01	P02	P03	P04	P05	P06	P07	P08	P09	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19	P20	P21	P22	P23	P24	P25	P26	P27	P28	P29	P30	P31	P32			
barleys	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			
B1	x	x				x					x				x					x				x				x								
B2	x	x					x				x					x					x					x										
B3	x				x	x								x							x	x					x							x		
B4	x			x								x					x	x							x									x		
B5	x				x							x									x														x	
B6	x			x								x									x	x					x							x		
B7	x		x								x																								x	
B8	x			x																																x

**Fig. A5.** Experimental design arranged as an incomplete factorial. Every 'x' within the green zone represents one specific mixture. 'x's within a square of 8 x 8 were distributed in a Latin-square manner, in a way that only two specific treatments are realized, ensuring balance between barley and pea. 'x's within the blue and yellow zone represent barley and pea pure stands, respectively.

## 8.6. Figures. Example experimental layout and aerial picture



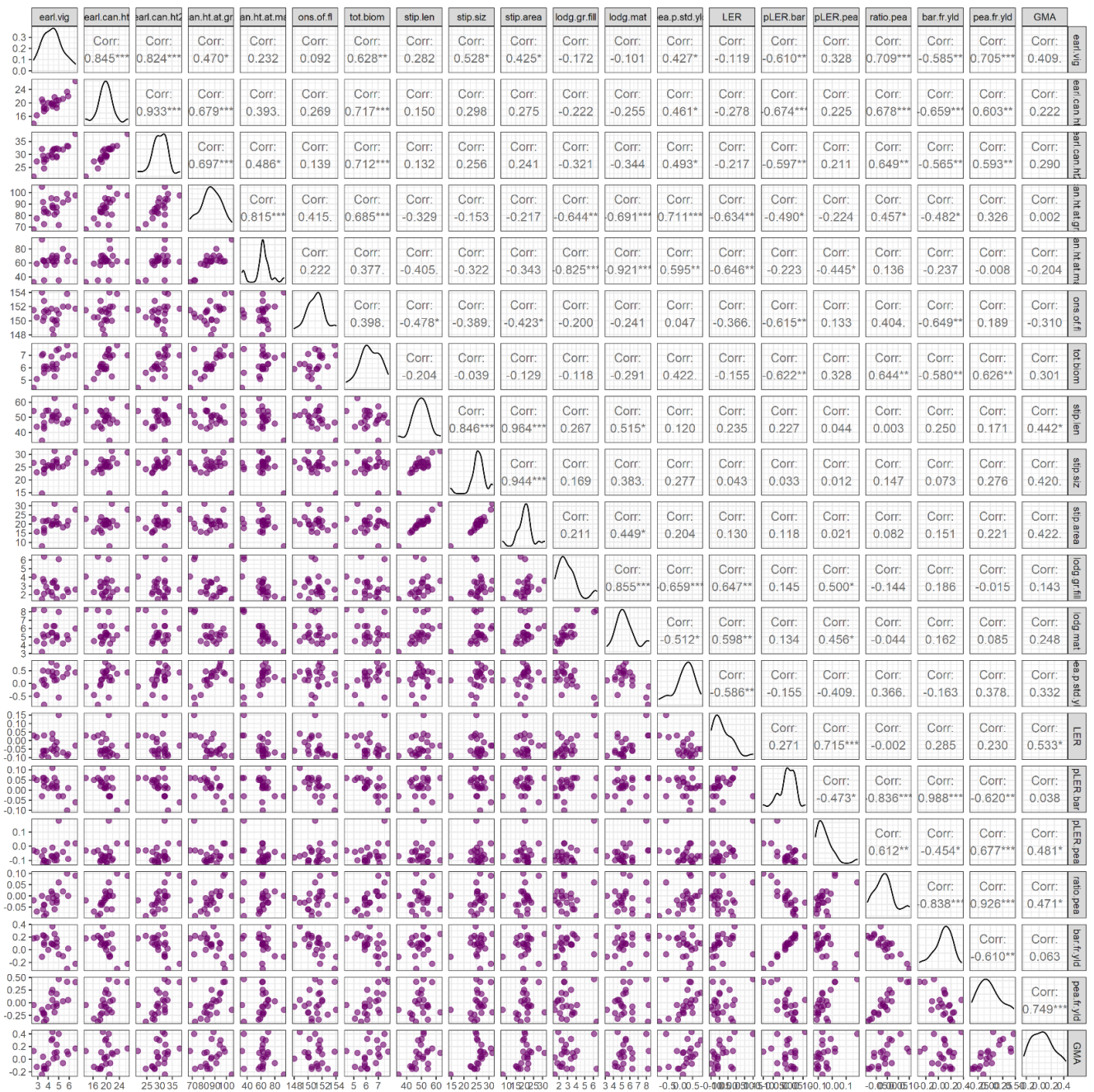
**Fig. A6.** (A) Example for one experimental layout (Fislisbach 2019). On the trial plan, the two replications are separated by the thick black line; within each replication treatments were randomized, with dark grey, light gray and white rectangles representing barley pure stands, pea pure stands and mixtures respectively. (B) An aerial image of the same trial taken seven weeks after sowing.

## 8.7. Table. Overview measurements

**Table A7.** Overview over the data structure of the 12 traits measured in pure stand in the four experimental environments. Every 'x' represents a measuring environment, '(x)' indicate an environment in which only a subset of all genotypes has been measured.

Trait	Experimental environment			
	2018		2019	
	Fislisbach	Uster	Fislisbach	Uster
Early vigor	x	x	x	x
Early canopy height	x		x	x
Early canopy height (2nd date)	x	x	x	x
Canopy height at grain filling	x	x	x	x
Canopy height at maturity		x		x
Onset of flowering pea	x	x	x	
Plant biomass	x	x	x	
Stipule length	x		(x)	
Stipule size	x		(x)	
Stipule area	x		(x)	
Lodging at grain filling	x	x	x	x
Lodging at maturity		x	x	x

## 8.8. Figure. Correlation matrix traits



**Fig. A8.** Correlation-matrix of twelve putative key traits, measured in pea pure stands, and eight yield-related traits of pea. Each dot represents the measurement of one pea genotype, averaged over all experiments (yield related traits: emmeans). The dataset did not contain the GMs due to their high intra-treatment variability in traits, and not P28 due to its atypical phenotype (highly prone to lodging).

## 8.9. Table. Emmeans of trait measurements

**Table A9 (following page).** Trait measurement of four putative key traits of pea for mixed cropping. Effects sum to zero and relate to the given average. See materials and methods for a description of trait measurements and trait-pre-selection procedure.

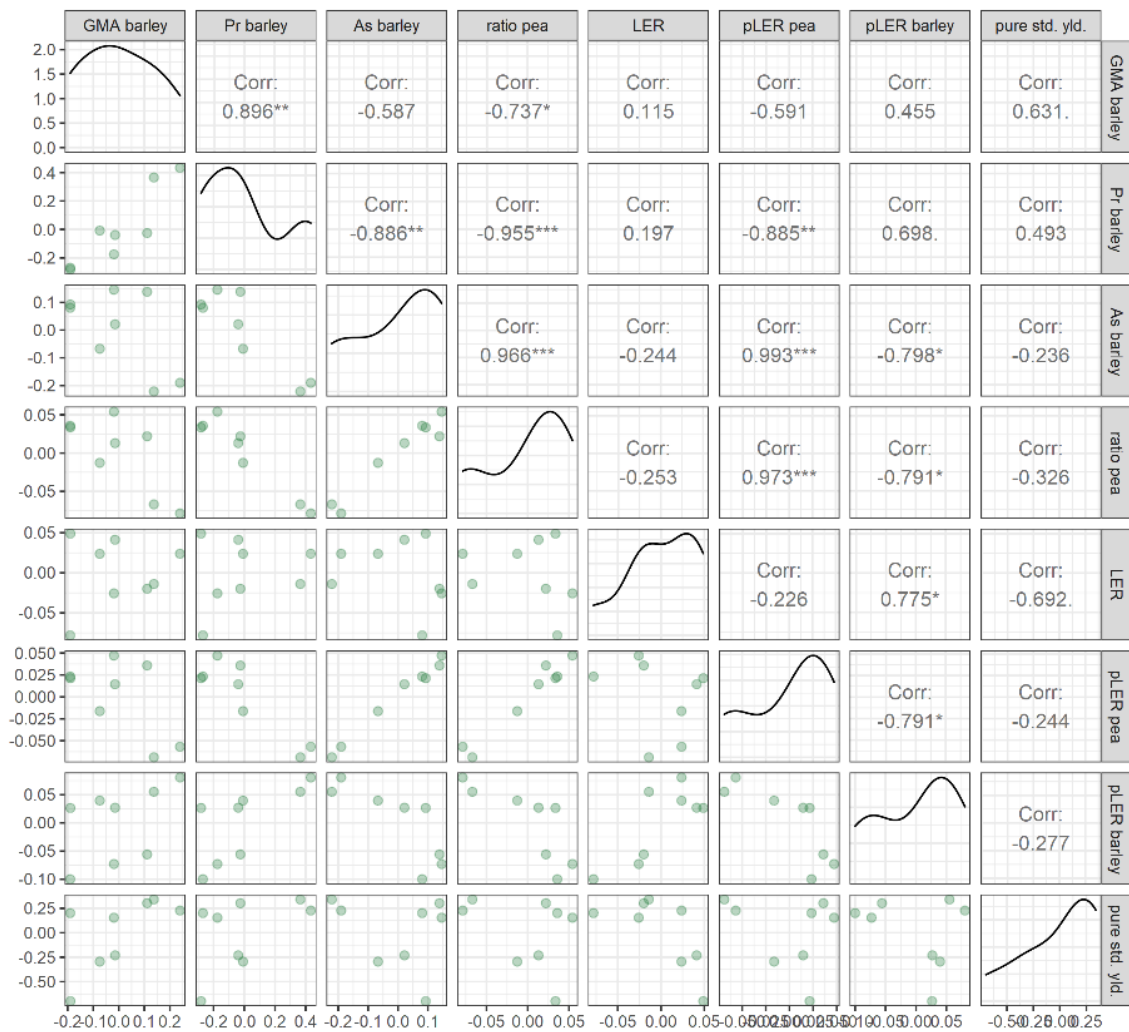
Pea genotype	Plant size and leafiness <sup>1</sup>		Early vigor [1-9]	Onset of flowering [days after 1st of Jan.]	Biomass [1-9]	Stipule length [mm]	Early canopy height (1st measurement) [cm]	Early canopy height (2nd measurement) [cm]	Canopy height at grain filling [cm]	Canopy height at maturity <sup>3</sup> [cm]	Lodging at grain filling [1-9]	Lodging at maturity [1-9]
P01-SG-L 7647	L	s. leafl.	-1.0 **	2.8 ***	0.6	-15.4 ***	1.0	2.0 *	16.8 ***	28.7	-1.9 **	-2.8 ***
P02-Impuls	L	s. leafl.	2.3 ***	0.5	0.6	7.4 *	6.5 ***	7.8 ***	9.3 **	-3.0	-0.8	0.0
P03-Astronaute	S	s. leafl.	0.2	-0.2	-0.5	-2.4	-0.5	-1.4	-1.1	2.6	-1.7 *	0.0
P05-Navarro	S	s. leafl.	0.1	-3.2 ***	-0.6	1.1	-0.5	1.9 *	0.8	-6.4	0.0	0.4
P06-Gambit	L	s. leafl.	1.4 ***	2.7 ***	1.3 ***	-3.4	3.4 **	3.1 ***	10.5 ***	3.7	-0.8	-0.1
P07-Angelus	S	s. leafl.	-0.7 *	1.7 **	-0.3	-6.1 .	-1.6	-2.0 *	-1.3	3.7	0.1	-1.0
P08-Salamanca	L	s. leafl.	0.4	0.3	0.9 **	-0.3	0.7	2.1 *	7.1 *	3.7	-0.7	-0.8
P10-Rocket	M	s. leafl.	-0.9 **	-1.2 *	0.7 .	1.3	0.2	-0.6	3.4	-4.7	0.3	-0.6
P11-Karpate	S	s. leafl.	0.4	-1.7 **	-0.2	3.2	-1.5	-1.2	-4.3	18.7	-0.5	-1.0
P12-Kayanne	M	s. leafl.	0.0	-1.0 .	-0.3	0.2	0.0	-1.2	-0.2	-1.4	0.0	-0.5
P13-Mytic	S	s. leafl.	-0.7 *	0.2	-0.6	-8.2 ***	-1.3	-3.0 **	-4.3	6.2	-1.3 .	-1.8 *
P14-Protecta	L	leafy	0.6 *	-0.2	1.1 **	0.8	0.9	1.9 *	-11.6 *	-21.4	2.7 ***	2.1 *
P18-Alvesta	S	s. leafl.	-0.4	0.0	-0.6	3.8	0.0	-0.6	-6.4 *	11.2	-1.0	-0.8
P19-Bockros	M	s. leafl.	0.3	-1.2 *	-1.2 ***	12.0 ***	1.7	2.0 *	-7.2 *	1.2	0.2	0.4
P20-Volt	M	s. leafl.	0.0	-2.3 ***	-0.2	0.8	-1.0	1.1	0.5	-3.9	-1.5 *	-1.5 .
P21-Biathlon	S	s. leafl.	-1.7 ***	0.3	-2.1 ***	4.2	-6.1 ***	-8.6 ***	-18.7 ***	-23.9	0.7	2.4 **
P22-Tip	M	s. leafl.	1.0 **	2.3 ***	0.3	-4.1	1.2	1.8 .	3.0	18.7	-1.7 *	-1.8 *
P24-Peps	L	s. leafl.	1.5 ***	0.8	0.6	-1.4	2.0	3.0 **	5.5 .	13.7	-1.2 .	-1.3 .
P25-Karioka	M	s. leafl.	-0.1	0.5	0.4	-2.1	-0.3	-0.2	6.8 *	11.2	-1.2 .	-0.5
P27-Milwa	S	s. leafl.	-1.5 ***	-0.7	-1.3 ***	-6.3	-3.6 **	-2.9 **	-10.7 ***	11.2	-1.2 .	-1.5 .
P28-Florida	L	leafy	2.1 ***	0.7	1.3 ***	3.8	4.0 **	4.0 ***	-15.7 <sup>2</sup>	-18.9	5.2 ***	3.0 ***
P29-Natura	S	leafy	-0.7 *	-0.7	-0.8 *	-3.6	-2.5 .	-5.5 ***	-13.0 *	-21.4	3.0 ***	2.2 **
P31-Starter	S	s. leafl.	-0.6 .	0.3	-0.3	3.7	-1.8	-2.1 *	-3.5	-6.4	-0.5	0.4
P09-GM-short	M	both	-1.2 ***	-1.4 *	-0.4	0.4	-1.1	-1.7 .	-2.6	8.7	-1.2 .	0.2
P26-GM-long	L	both	-0.9 **	0.8	0.6		0.7	1.3	14.5 ***	-8.9	1.2 .	2.2 **
P04-GM-semi-leafless	M	s. leafl.	-0.6 .	-1.2 .	0.1	-4.8	-0.6	-0.7	0.5	-6.4	-1.3 .	-0.6
P32-GM-leafy	L	leafy	0.4	0.8	0.8 *	15.9 ***	-0.5	-0.4	6.4	-16.4	4.7 ***	3.0 ***
min. (effect)			-1.7	-3.2	-2.1	-15.4	-6.1	-8.6	-18.7	-23.9	-1.9	-2.8
mean (effect)			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
max. (effect)			2.3	2.8	1.3	15.9	6.5	7.8	16.8	28.7	5.2	3.0
<b>average</b>			<b>4.3</b>	<b>150.7</b>	<b>6.4</b>	<b>50.9</b>	<b>24.0</b>	<b>27.8</b>	<b>83.2</b>	<b>65.2</b>	<b>3.4</b>	<b>6.0</b>

<sup>1</sup>plant sizes: S(hort), M(edium) and L(ong); leafiness: semi-leafless ('s. leafl.') or 'leafy', see main text, <sup>2</sup> data only from one exp. environment available, no t.test possible

<sup>3</sup>genotypic effects not significant (high measurement error)

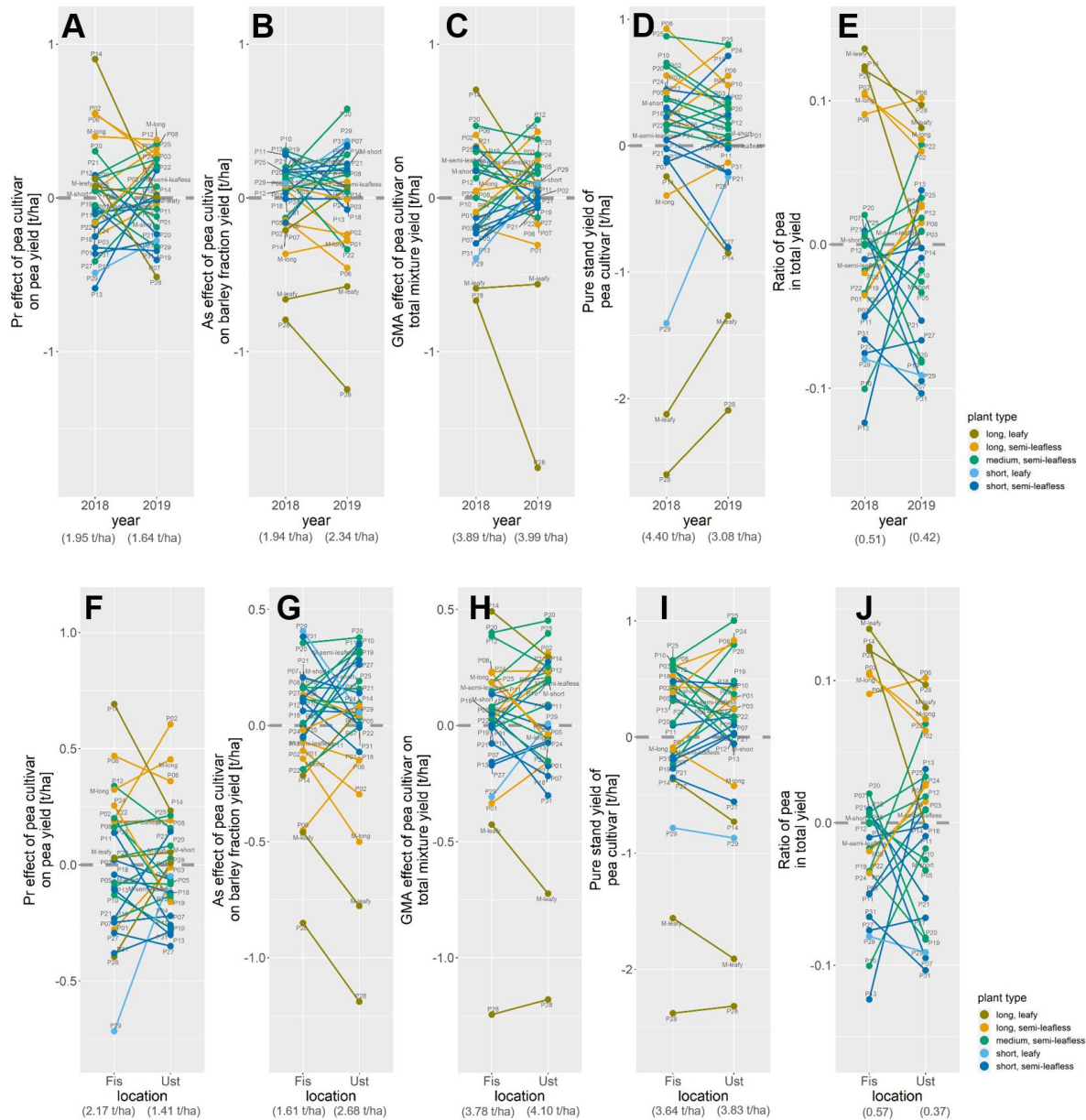


## 8.10. Figure. Correlation matrix yield parameters barley



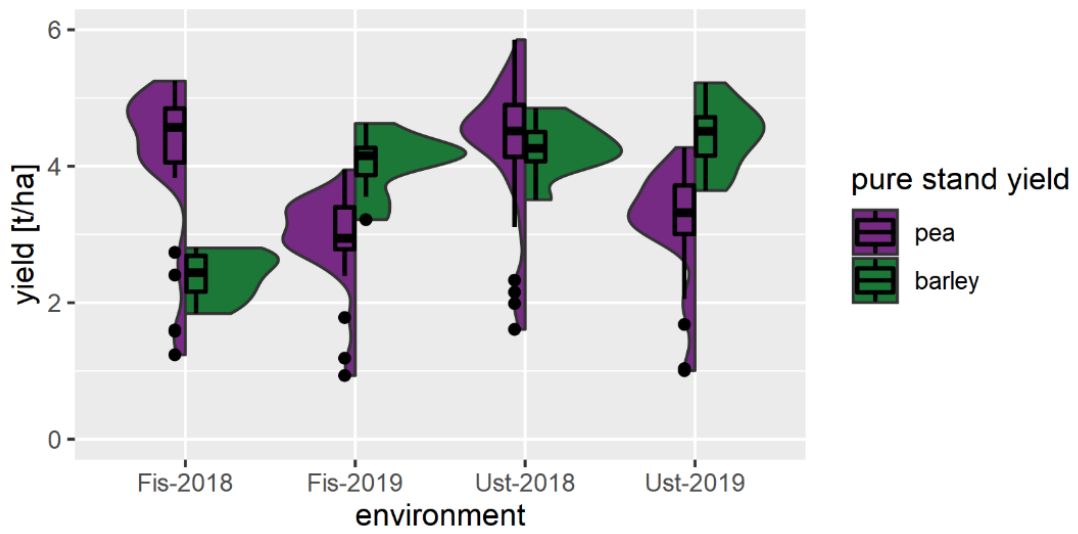
**Fig. A10.** Correlation matrix of yield related parameters of barley. Each dot represents one barley genotype (see Table A7, p. 93).

## 8.11. Figures. Interaction plots years and locations pea



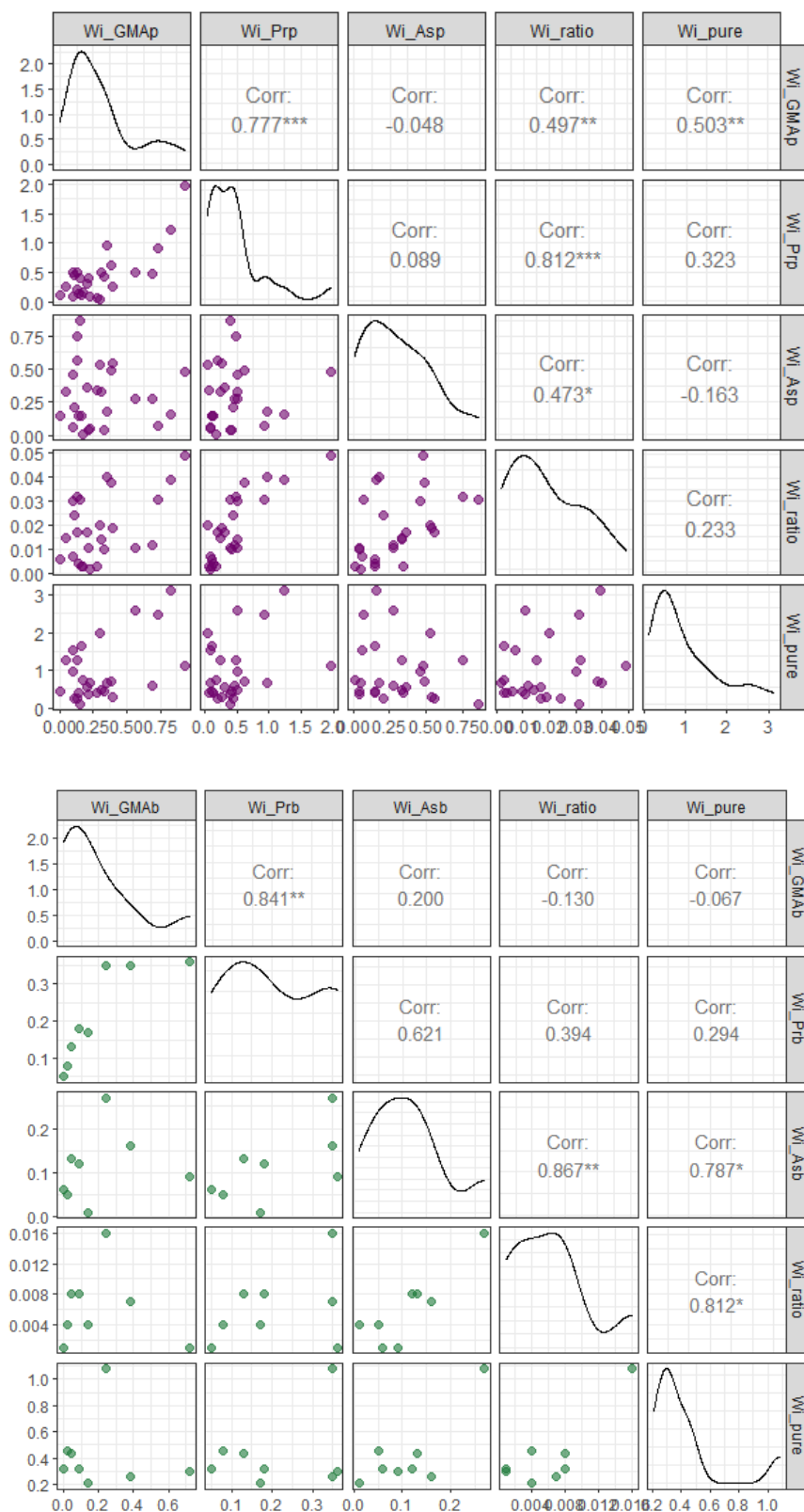
**Fig. A11.** Genotype x year interaction-plots of pea for Pr- and As-effects (A and B, respectively), GMA (C), pure stand yield (D), and ratio of pea in total yield (E) for the years 2018 and 2019. All connecting lines that are not horizontal indicate a G x year interaction. Numbers below the years indicate the averages for the corresponding year. Genotype x location interaction-plots of pea for Pr- and As-effects (F and G, respectively), GMA (H), pure stand yield (I), and ratio of pea in total yield (J) for the locations Fislisbach ("Fis") and Uster ("Ust"). All connecting lines that are not horizontal indicate a G x location interaction. Numbers below the locations indicate the averages for the corresponding location.

## 8.12. Figure. Violin plots of pure stand yields over environments



**Fig. A12.** Pure stand yields in the four experimental environments.

### 8.13. Figures. Correlation matrices of Wi of pea and barley parameters



**Fig. A13 A.** Correlation matrix of Ecovalences of pea (top) and of Ecovalences of barley (bottom; see also Table A7, p. 93).

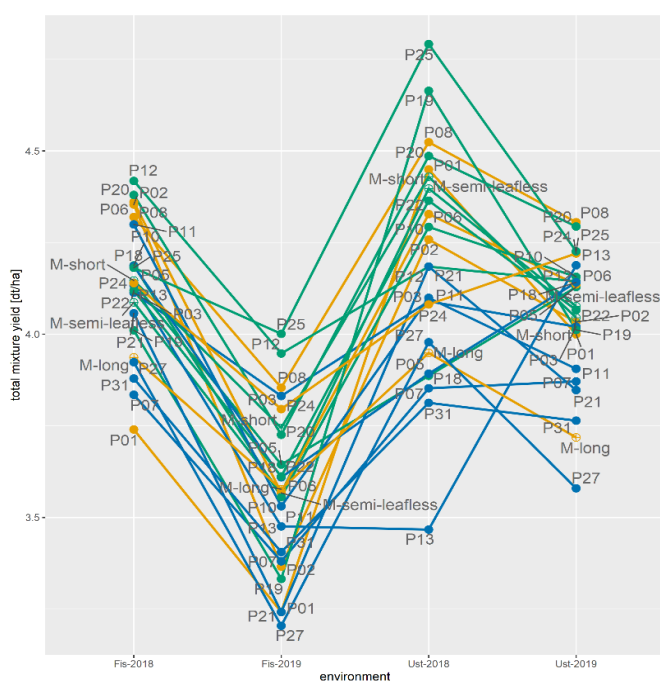
## 8.14. Table. Anova table for genotype x culture interaction

**Table A14.** Analysis of variance for genotype ('gen.') and culture (levels: 'pure' or 'mixed') effects and genotype x culture interactions for different dependent yield-variables.

Dependent variable	Independent variables	df	SS	MS	F value	Pr(>F)
yield (total yield in mixture)	pea gen.	26	101.9	3.9	10.1	< 0.0001 ***
	culture	1	5.8	5.8	15.0	0.0001 ***
	pea gen.:culture	26	34.5	1.3	3.4	< 0.0001 ***
	Residuals	583	227.3	0.4		
yield (pea fraction yield in mixture)	pea gen.	26	68.0	2.6	4.9	< 0.0001 ***
	culture	1	525.0	525.0	990.1	< 0.0001 ***
	pea gen.:culture	26	60.9	2.3	4.4	< 0.0001 ***
	Residuals	579	307.0	0.5		
yield (total yield in mixture)	barley gen.	7	6.3	0.9	2.5	0.0155 *
	culture	1	1.2	1.2	3.3	0.0716 .
	barley gen.:culture	7	8.0	1.1	3.2	0.0027 **
	Residuals	474	170.7	0.4		
yield (barley fraction yield in mixture)	barley gen.	7	23.3	3.3	5.5	< 0.0001 ***
	culture	1	148.7	148.7	247.9	< 0.0001 ***
	barley gen.:culture	7	8.3	1.2	2.0	0.0564 .
	Residuals	469	281.3	0.6		

. < 0.1 \* < 0.05 \*\* < 0.01 \*\*\* < 0.001

## 8.15. Figure. Interaction plot of total yield by environment



**Fig. A15.** Interaction-plot for total mixture yield of all semi-leafless genotypes.

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