

RESEARCH PAPER

Intercropping drives plant phenotypic plasticity and changes in functional trait space



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Abstract

The relevance of intercropping, where two or more crop species are simultaneously grown on the same land space, is growing due to its potential for improving resource use and maintaining stable yields under variable weather conditions. However, the actual growth of intercropped species may differ resulting from the idiosyncratic effect of crop diversity, and with this, the realized benefits from intercrops are found to depend critically on the cultivar, species, management and environmental conditions. This study aimed to apply a trait-based approach, in which ecological niche spaces are defined through n -dimensional hypervolumes, to identify the contribution of species/cultivar, cultivation design (sole crop or intercrop) and management (low or high fertilization) to the trait diversity of four crop species, pea-barley and faba bean-wheat, when grown as sole crops and intercrops. Four traits were used as trait axes for the trait space analysis: canopy height, shoot biomass, tiller/node number, and grain yield. We found that trait spaces differed with crop species and cultivars, and whether they were grown as intercrops or sole crops. Trait spaces differed between high and low fertilization only for the cereals grown in the more productive site (i.e. Denmark). Species grown as intercrops had larger volumes than when grown as sole crops, as a result of trait plasticity. This response to intercropping was apparent in almost all the species grown in Sweden and Denmark, except for wheat in Denmark. The study demonstrated that individual species responded to intercropping compared to sole cropping through the plasticity of traits, which influenced the shape of the hypervolumes to divide up the trait space between the species. The findings are important in illustrating the plastic responses of arable crops, which are relevant for understanding the productivity of species grown in intercrops as compared to sole crops.

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Introduction

The deliberate inclusion or maintenance of plant diversity in crop fields is beneficial in maintaining crop yields and reducing negative environmental impacts (Li et al., 2009; Lowry et al., 2020). Species diversity in intercrops is particularly important in intensively managed crop fields under a scenario of increasing biodiversity loss or climate change (Himanen, Mäkinen, Rimhanen, & Savikko, 2016). Intercropping is a practical means of crop field diversification that involves growing two or more crop species together at the same time. Different crop species typically have differences in their functional traits, allowing spatial or temporal sharing of niche space on the same piece of land (Brooker et al., 2015; Li et al., 2020). Here, functional traits are the morphological, physiological or phenological plant features that can be measured at a whole-plant level and which are capable of influencing plant fitness (Litrico & Violle, 2015).

Plant functional traits have an indirect influence on the ecological niche occupied by plants. In this study, *trait space* is defined as a multidimensional shape that derives its unique properties from the difference or similarity of the functional traits that constitute the axes. According to ecological niche theory, the more the functional trait values of the intercrop components differ within the functional trait space suitable for that environment, the more productive the mixture is, as component species are then able to utilize resources from different niches (Li et al., 2006; Lowry et al., 2020; Zhu, van der Werf, Anten, Vos, & Evers, 2015). Resource utilization of different niches ensures that more complete resource exploitation is achieved by the community components due to niche complementarity and thus weaker interspecific competition (Brooker et al., 2015).

Functional trait variation can be caused by genetic differences between species and cultivars as well as by trait plasticity (Dahlin, Kiær, Bergkvist, Weih, & Ninkovic, 2019; Zhu et al., 2015), with trait (or *phenotypic*) plasticity being the ability of a single genotype to express different trait values under different environmental conditions (Arnold, Kruuk, & Nicotra, 2019; Bradshaw, 1965). In the context of intercrops, the environment experienced by a crop genotype is shaped by local soil and weather conditions, prevalent biotic elements, as well as field management, including the establishment of other plant species as part of the mixture. In addition, it is not uncommon for some species grown as intercrop to have different trait values compared to a cultivation as sole crop, resulting from competitive relations which render some species to dominate (outcompete) others. The differential expression of phenotypes in different environments has the potential for shaping plant-plant interaction in intercrops. For example, growing different species as an intercrop can alter light capture within the canopy, as exemplified in wheat-maize intercrops (Zhu et al., 2015). The different phenotype expressions have implications for species-specific resource use even though only marginal differences in total resource capture are achieved in some cases (Li et al., 2021).

Here, we focus on four functional traits related to above-ground resource acquisition: Canopy height, number of tillers (cereals) or nodes (legumes), shoot biomass, and grain yield. In intercrops, the canopy heights of the intercropped species play a significant role in the interaction between them, especially for light interception, which together with the distribution of nitrogen over the canopy can affect light use efficiency (Berghuijs et al., 2020; Gou et al., 2017). Moreover, the light conditions below the canopy are known to influence tillering in cereals and branching in dicotyledonous plants such as legumes (Deregibus, Sanchez, & Casal, 1983), and we expect the tillering or node forming potential of a given species or cultivar to be a strong driver for its capacity to make use of the increasing resource (light, nutrients) availability that can result from intercropping of complementary species. Compared to node formation in legumes, the vegetative and structural nature of tillers provides a greater degree of plasticity (e.g. in tiller number) in cereals (Zhang, Wang, Ma, Yang, & Atkin, 2015). We therefore expect that the magnitude of tillering variation in cereal species is greater than node formation in legume species. The outcome of differential canopy heights and tiller or node setting capacities of the component species grown in an intercrop is reflected by the shoot biomass and grain yields of the component species. Taken together, the selected traits are therefore considered especially relevant in unravelling the specific change in interactions among intercropped species as compared to the same species grown as sole crops, resulting from trait plasticity.

We combine a multidimensional trait-based approach with multivariate analysis to evaluate the contribution of different functional traits to the interaction and performance in intercrops, defined via n -dimensional hypervolumes (Blonder, 2018; Blonder, Lamanna, Violle, & Enquist, 2014). This approach has previously been applied in managed sole crops and intercrops (Ajala, Jäck, Vico, & Weih, 2021). The present study aimed to understand the processes that generate the overall trait diversity observed in the intercrops, and to identify the contribution of species/cultivar, cultivation design (sole crop or intercrop) and management (low or high fertilization) to the trait diversity. Intercrops are specifically known to have enhanced resource sharing and niche complementarity due to the architectural differences in the species, allowing for a yield advantage to the intercrops compared to the sole-grown crops (Brooker et al., 2015; Hauggaard-Nielsen, Ambus, & Jensen, 2001). We hypothesize that: *H1*) Plant trait values of individual species differ when grown as an intercrop and/or under different fertilization levels, with plants producing more tillers/nodes, taller canopies, and more shoot biomass and grain yield in the intercrop compared to the corresponding sole crops due to resource complementarity. As a result, species grown as intercrop components display larger trait hypervolumes due to larger variation within traits than the same species grown as a sole crop; and *H2*) The interaction between species/cultivar identity and growth conditions (as driven by different weather and soils) cause a shift in hypervolume dimensions between the sites due to plasticity of plant functional traits.

Table 1. Mean trait values for different crop species grown as sole crops or intercrops in Denmark and Sweden pooled for 2017 and 2018 growing seasons. All measurements were taken around crop flowering except the grain yield which was measured at maturity. Values in parenthesis are standard errors.

Crop species	Denmark			Sweden				
	Canopy height (cm)	Tiller or node number (plant ⁻¹)	Shoot biomass (g m ⁻²)	Grain yield (g m ⁻²)	Canopy height (cm)	Tiller or node number (plant ⁻¹)	Shoot biomass (g m ⁻²)	Grain yield (g m ⁻²)
<i>Pea-barley</i>								
Pea sole	93.2 (2.1)	15.50 (0.5)	394 (14.1)	380 (8.2)	36.4 (3.0)	15.70 (0.5)	236 (7.4)	116 (6.2)
Pea intercrop	81.1 (1.4)	13.20 (0.5)	446 (18.3)	375 (18.5)	30.9 (1.3)	14.60 (0.3)	169 (7.3)	76 (4.4)
Barley sole	61.7 (1.5)	3.18 (0.2)	238 (18.9)	222 (12.1)	32.5 (1.9)	2.02 (0.1)	219 (13.5)	191 (15.1)
Barley intercrop	65.4 (1.7)	3.61 (0.2)	263 (19.1)	271 (18.4)	33.6 (1.5)	2.16 (0.1)	297 (15.1)	276 (17.1)
<i>Faba bean-wheat</i>								
Faba bean sole	96.6 (1.6)	8.46 (0.1)	333 (9.2)	401 (24.2)	30.2 (2.1)	18.20 (0.4)	154 (4.6)	166 (7.1)
Faba bean intercrop	74.7 (1.6)	7.73 (0.1)	279 (19.0)	286 (23.0)	29.3 (1.0)	16.80 (0.2)	224 (14.4)	247 (17.0)
Wheat sole	58.6 (2.4)	2.23 (0.1)	276 (14.0)	214 (11.0)	37.5 (2.7)	1.47 (0.1)	212 (8.6)	213 (10.9)
Wheat intercrop	57.3 (2.5)	2.80 (0.1)	328 (13.5)	310 (12.2)	35.8 (2.0)	1.68 (0.0)	190 (8.6)	160 (9.7)

Materials and methods

Experimental sites

The experiments were conducted in two sites, in Uppsala, Sweden (59° 50' 6" N 15° 42' 0" E) and Taastrup, Denmark (56° 40' 7" N 12° 18' 20" E) in the summer seasons of 2017 and 2018. The sites were chosen because the weather conditions are relatively similar and therefore favorable for the cultivation of similar genotypes/cultivars. A summary of weather and management conditions is presented in Table 1 by Weih et al. (2021).

Plant material and experimental design

Pea (*Pisum sativum* L.) – spring barley (*Hordeum vulgare* L.), and spring wheat (*Triticum aestivum* L.) – faba bean (*Vicia faba* L.) species combinations were field-grown in Sweden and Denmark. For details on the cultivars, see Appendix A: Table S1A and S1B). In Sweden, fields were sown on 5 May and harvested on 16 August in 2017; and sown on 30 April and harvested on 16 August in 2018. Seeds were sown at a depth of 6 cm using a tractor-mounted mechanical plot drill. The Danish fields were sown relatively earlier than in Sweden, i.e., 7 April and harvested 6 September in 2017; and sown on 18 April and harvested on 8 August in 2018. Within each site and species combination, each cultivar (cv.) of each species was grown as a sole crop and as a component in two-species intercrop mixtures with 50:50 proportions in a replacement design. The species in the intercrops were full mixtures, mixed within rows. For the trait space analysis, cultivars were selected such that each species combination had at least one cultivar included in both sites, except for a few cases to allow creation and comparison of hypervolumes with similar numbers of observations.

In each site and year, the plots were arranged in a split-plot design with four replicates having ‘low’ and ‘high’ N treatments as main plots and cultivars grown as a sole crop or intercrop as subplots. For the low N treatment, 0 and 20 kg N ha⁻¹ was applied in Sweden and Denmark, respectively. In the high N treatment, NPK fertilizer with N in the form of NH₄NO₃ was applied (pea-barley; 90 kg N ha⁻¹, 15 kg P ha⁻¹ and 29 kg K ha⁻¹, and in faba bean-wheat; 40 kg N ha⁻¹, 24 kg P ha⁻¹ and 46 kg K ha⁻¹.) in the Swedish plots; and as 60 kg N ha⁻¹ in both pea-barley and faba bean-wheat species combinations in Denmark.

Trait assessment and trait space analysis

Canopy height, shoot biomass and grain yield were assessed on plot level for each species grown as sole crop or intercrop, whereas number of tillers and nodes was assessed at individual plant level. These four traits are commonly used to evaluate crop performance in many agronomic studies. A detailed measurement protocol is included in the

Appendix A. For traits assessed per area, the expected biomass and grain yields in the mixtures were calculated from $E_i = p_i M_i$ (Loreau, 1998) in which E_i is the expected biomass or grain yield in the intercrop, p_i is the proportion of each species in the mixture (0.5 in this study), and M_i is the biomass or grain yield in the sole crop. Comparisons for yields in the sole crop and intercrop were based on the expected yields. Traits were assessed at the flowering stage, except for grain yield, which was assessed at crop maturity.

Trait space was analyzed using the Gaussian kernel density estimation method of the ‘hypervolume’ R package (Blonder, 2018; Blonder & Harris, 2018; Blonder et al., 2014). Briefly, this method employs n -dimensional kernel density estimation (where n is the number of traits) on a grid of random points around the data; hypervolumes are then defined by slicing the kernel densities and a process termed importance weighting. For details see (Blonder et al., 2014). The method was used to determine how the hypervolumes and the measures of volume overlap differ in the sole and intercrops, and trial sites. All measured data were log-transformed before analysis. For each species, data were grouped per cultivar, and analysis was done for each intercropped species pair. Simulations based on the mean and standard deviations of the measured data were used to generate random variables, using the replicate() function in R (Muldoon, 2018). The generated replicates created variability and permitted the testing of statistical significance through the analysis of variance among the different indices between treatments. The hypervolumes were then constructed based on the simulated values. For each crop species, hypervolumes were generated for each cultivar grown in sole crop (A) and intercrop (B), respectively, and the hypervolume units were expressed as SD^4 to denote the product of the axes’ standard deviations. Bar graphs were used to represent the four metrics that describe hypervolume properties i.e., hypervolume size, centroid distance, Jaccard similarity and the unique volume fraction. The calculation flow is presented in the Appendix (see Appendix A: Fig. S1). Hypervolume size represents the multidimensional space within a specific hypervolume, e.g. the sole crop hypervolume A. The centroid distance measures the Euclidean distance between the centroids (i.e. centres) of hypervolumes A and B. Jaccard similarity represents the intersection area of the two hypervolumes divided by their union area ($|A \cap B| / |A \cup B|$). The unique volume fraction is the unique area component of a given hypervolume divided by its hypervolume size, e.g., for sole crop (A) is given by unique fraction A /hypervolume A.

Statistical analysis

All statistical analyses were performed in R version 4.0.2 (R Core Team, 2020). Results from the hypervolume analysis for each crop species grown as sole crop or intercrop were analyzed separately for most of the attributes investigated except for centroid distance and Jaccard similarity where the analysis required data from both the sole crops and intercrops. Data

normality assumptions were evaluated visually on plots, and data sets that did not meet the assumptions were log-transformed and the population means in the sole crop and intercrop compared using Tukey’s test. Data not resolved by transformation were analyzed through the non-parametric Kruskal-Wallis tests (Kruskal & Wallis, 1952). This was done for hypervolume size and unique volume fraction for barley and faba bean in Sweden, and unique volume fraction for faba bean in Denmark. Mean trait values were analyzed through a linear mixed effects model [nlme Package: ‘lme’ by Pinheiro et al. (2017)] with Year, Intercropping effect, Management, and their interactions as fixed effects, and Management nested within blocks as random effect. Trait plasticity was analysed with the four traits combined using permutational multivariate analysis of variance, as implemented in the *vegan* R package (Oksanen et al., 2018). The distance matrices were used to determine the dissimilarity among the different sources of variation (Intercropping effect, Cultivar, Management and their interactions). Through this, we determined trait responses to the different treatments based on the pairwise Euclidean distances.

Results

Mean trait values of crop species depend on the diversity level

We found differences in mean trait values among the crop species, and when the crops were grown as sole crop or intercrop (Table 1, Appendix A: Tables S2, S3, S4). For traits assessed per plant (i.e., tiller and node number), both legume species generally set fewer nodes in intercrops than in sole crops at both sites (9.4% and 17.4% less for faba bean and pea, respectively in Denmark, and 24.6% and 7.5% less for faba bean and pea, respectively in Sweden). The cereals produced more tillers in the intercrop than in the sole crop (i.e., 13.5% and 25.5% more in the intercrop of barley and wheat, respectively, in Denmark. In Sweden, wheat had 14% more tillers in the intercrop than in the sole crop while no difference was observed for barley. Wheat and both legume species grown in Denmark as intercrops had shorter canopies than in the sole crops. In Sweden, pea had shorter canopies in the intercrop than in the sole crop while for faba bean, wheat and barley intercropping had no effect on the canopy height (see Appendix A: Table S2, S3, S4). For traits assessed per area (i.e., shoot biomass and grain yield), in Denmark, both cereals produced more shoot biomass (263 g m⁻²; 10.5% more, and 328 g m⁻²; 18.8% more for barley and wheat respectively) and grain yield (271 g m⁻²; 22.0% more, and 310 g m⁻²; 44.8% more for barley and wheat, respectively) in the intercrop than the sole crop. In Sweden, barley produced more shoot biomass and grain yield in the intercrop than in the sole crop, whereas wheat produced less shoot biomass and grain yield in the intercrop than in the sole crop. For the legumes, pea consistently had less grain yield in the intercrop than in the sole crop in both sites,

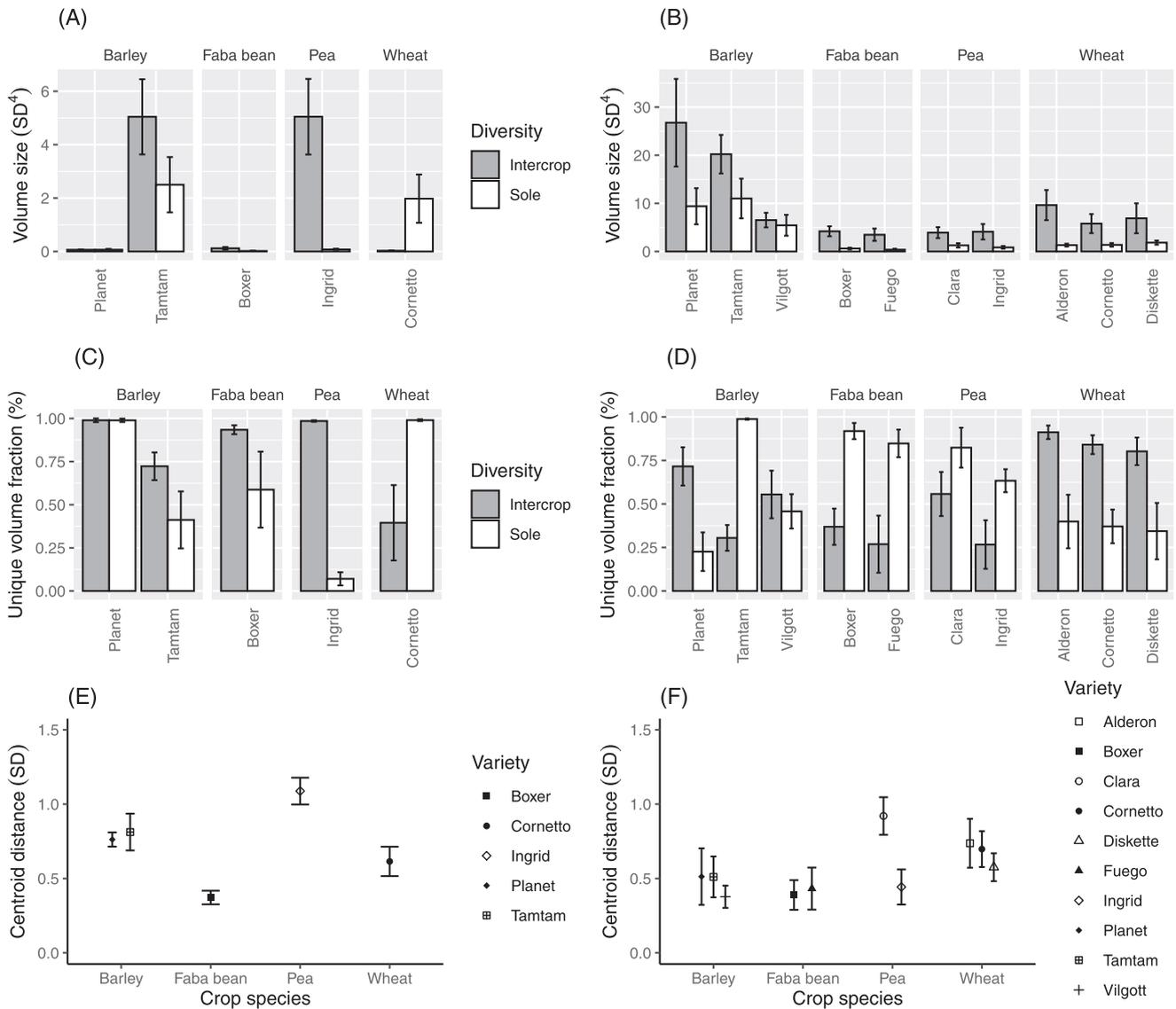


Fig. 1. Trait space analysis for different cultivars of pea, barley, faba bean and wheat, grown as sole crops and intercrops in Copenhagen, Denmark ((A), (C) and (E)) and in Uppsala, Sweden ((B), (D) and (F)). The cereals were grown with a similar legume partner and vice versa for the legumes at each of the sites. All wheat cultivars were grown with faba bean cv. Fuego while faba bean cultivars with wheat cv. Cornetto. Barley cultivars were grown with pea cv. Ingrid while pea cultivars were grown with barley cv. Tamtam. Data were pooled per cultivar of each crop species grown in 2017 and 2018. The height of the bars measures the variability of the 4-dimensional traits and the error bars represent mean ± 1SD, accounting for the variability in different volumes created.

whilst faba bean showed a similar pattern only in Denmark. In Sweden, faba bean produced more shoot biomass and grain yield in the intercrop than in the sole crop.

Trait spaces of species expand when intercropped

Growing the crop species as sole or intercrops resulted in differing trait space values, with all species and cultivars having larger trait spaces in the intercrops than the sole crops in both sites, except for wheat in Denmark, which had a larger hypervolume in the sole crop than in the intercrop (Fig. 1, Table 2). The hypervolumes for

barley cultivars grown in Sweden as intercrops were specifically higher than all the other species (cv. Planet had the highest hypervolume of 26.80 SD⁴), implying larger variability in trait values. The unique volume fractions in Denmark followed a similar trend as the hypervolumes, i.e., larger in the intercrops except for wheat, while the pattern was variable in Sweden. The legumes and cereals had consistently higher unique volume fractions in the sole crops and intercrops, respectively, except for barley cv. Tamtam (Fig. 1C and D). The distance between hypervolume centroids only differed among pea and wheat cultivars grown in Sweden. Pea cv. Clara had a larger distance between centroids when grown in the sole crop

Table 2. . Analysis of Variance and Kruskal-Wallis tests for the effect of intercropping and cultivar on hypervolume size (SD^4) and unique hypervolume fraction (%) for pea, barley, faba bean and wheat grown as sole crops and as pea-barley and faba bean- wheat intercrops. Symbols show results with significant levels.

Crop/Cultivar	Denmark		Unique fraction		Sweden		Unique fraction	
	Hypervolume size F-value	P-value	F-value	P-value	Hypervolume size F-value	P-value	F-value	P-value
Pea								
Cultivar	-	-	-	-	2.46	0.125	43.53	<0.001***
Intercropping effect	-	<0.001***	-	<0.001***	128.14	<0.001***	75.59	<0.001***
C×IE	-	-	-	-	2.76	0.105	1.90	0.176
Barley								
Cultivar	1082.62	<0.001***	49.16	<0.001***	53.53	<0.001***	17.72	<0.001***
Intercropping effect	11.14	0.002**	12.91	<0.001***	55.67	<0.001***	1.56	0.216
C×IE	8.88	0.005**	12.88	<0.001***	7.70	0.001**	183.27	<0.001***
Faba bean								
Cultivar	-	-	-	-	11.41	0.002**	6.33	0.016*
Intercropping effect	82.70	<0.001***	-	<0.001***	304.72	<0.001***	275.25	<0.001***
C×IE	-	-	-	-	2.49	0.123	0.17	0.676
Wheat								
Cultivar	-	-	-	-	3.17	0.040*	2.96	0.060
Intercropping effect	371.80	<0.001***	-	<0.001***	338.31	<0.001***	296.89	<0.001***
C×IE	-	-	-	-	6.67	0.002**	0.35	0.704

Cells with F values denoted by hyphens (-) were analyzed using Kruskal-Wallis tests, hence no F values included. In cases where only one cultivar was used (especially in Denmark), the cells for cultivar and cultivar×Intercropping effect also have hyphens.

***= $P \leq 0.001$.

**= $P \leq 0.01$.

*= $P \leq 0.05$.

or intercrop and centroid distance for wheat cv. Alderon was larger than that of cv. Diskett (Fig. 1F).

Intercropped cereals and legumes had similar degree of trait plasticity

Both cereals and legumes showed a high overall degree of within-species trait plasticity ($p < 0.001$ in all cases), with barley grown as an intercrop in Sweden showing a marginal response (Table 3). A similar trend in the degree of overall trait plasticity was observed in Denmark, except in barley where intercropping had no influence on trait plasticity ($p = 0.548$). Trait plasticity variations between cultivars were different for all species grown in Sweden. In addition various interactions among cultivar, management and intercropping influenced trait plasticity especially in Sweden (Table 3). In Denmark, management, and its interaction with intercropping had a much stronger effect on trait plasticity under the more productive conditions at this site, while all cultivars of the different species produced similar levels of plasticity. High-input crop management (in the form of additional nutrient fertilization and pesticides) only had a significant effect on the degree of plasticity in the cereals grown in Denmark. Crop management (Management) consistently had significant effects on trait values of pea and faba bean grown as sole or intercrops (resulting in significant Intercropping by Management interaction; Table 3).

Large trait values were associated with small trait spaces under more productive conditions

For both cereal and legume species, the degree of overall trait plasticity differed when crops were grown in Denmark or Sweden (Table S5). Within each site, all crop species exhibited a shift in hypervolume dimensions when intercropped in each site (Fig. 2). In general, the more productive trials in Denmark generated larger trait values compared to Sweden, but exhibited smaller trait spaces (i.e. smaller trait variability) than in Sweden, especially when canopy height, shoot biomass and grain yield were compared by their hypervolume sizes (Fig. 3).

Discussion

Based on pea-barley and faba bean-wheat species combinations, we have shown that trait values and trait hypervolumes of different crop species are influenced by species diversity (i.e. when grown as intercrops or sole crops). These differences may be attributed to the plasticity of functional traits which in turn influences functional trait space of the crops. Apart from the intercropping effect, also cultivar choice was important particularly in the less productive conditions in Sweden, whilst management (fertilizer level) contributed more to trait plasticity in the generally more productive trial in Denmark. These results indicate that the

Table 3. Trait plasticity (in canopy height, tiller/node number, shoot biomass, and grain yield) for the different crop species and cultivars analysed through the use of a permutation distance matrix. Data for each cultivar were pooled for the 2017 and 2018 growing seasons. Symbols show results with significant levels.

Source of variation	Denmark		Sweden	
	F-value	P-value	F-value	P-value
Pea				
Cultivar	0.516	0.598	13.680	<0.001***
Intercropping effect	10.955	0.001**	25.561	<0.001***
Management	1.832	0.180	0.005	0.945
C×IE	3.159	0.011*	5.929	<0.001***
C×M	0.542	0.744	5.521	<0.001***
IE×M	4.547	0.005**	8.751	<0.001***
C×IE×M	1.513	0.144	2.816	0.002**
Barley				
Cultivar	2.391	0.054	21.820	<0.001***
Intercropping effect	0.362	0.548	4.082	0.045*
Management	15.916	0.000***	0.010	0.922
C×IE	3.328	0.001**	13.261	<0.001***
C×M	1.952	0.067	9.882	<0.001***
IE×M	5.901	0.001***	1.525	0.211
C×IE×M	1.513	0.111	5.820	<0.001***
Faba bean				
Cultivar	0.913	0.343	17.677	<0.001***
Intercropping effect	5.025	0.029*	123.150	<0.001***
Management	0.019	0.891	0.075	0.785
C×IE	1.443	0.240	34.239	<0.001***
C×M	0.595	0.621	7.044	<0.001***
IE×M	2.909	0.043*	35.009	<0.001***
C×IE×M	1.254	0.292	13.422	<0.001***
Wheat				
Cultivar	0.586	0.626	4.813	<0.001***
Intercropping effect	5.419	0.022*	45.956	<0.001***
Management	11.204	0.001**	0.319	0.573
C×IE	1.991	0.064	1.425	0.169
C×M	1.988	0.075	2.458	0.008**
IE×M	1.738	0.164	15.316	<0.001***
C×IE×M	0.936	0.521	1.219	0.245

***= $P \leq 0.001$.

**= $P \leq 0.01$.

*= $P \leq 0.05$.

response patterns affecting trait plasticity are complex and modulated by many factors, seemingly especially under low-resource conditions in our study which supports the findings by others (Perez-Ramos, Matias, Gomez-Aparicio, & Godoy, 2019). This pattern is reflected in our study by the many significant interactions on trait plasticity seen especially in the less productive conditions in Sweden.

Variation of functional traits when different species are intercropped

Except for a few cases, the legume species generally had lower trait values in the intercrops than in sole crops. For

example, intercropped pea set fewer nodes, grew shorter (hence low canopy), and produced less biomass and grain than in the sole crop. Considering that the plant population in the sole crops and intercrops were similar and based on the corrected values, the major driver for the difference could be competition for limited resources. Earlier studies have shown that in some cases, the legume performance in the intercrop is compromised by the cereal performance, especially in high-input systems where the cereals have a competitive advantage for N (Corre-Hellou, Fustec, & Crozat, 2006; Duchene, Vian, & Celette, 2017).

In line with the initial hypotheses, we found larger trait plasticity for cereals in some cases, i.e., increased tiller production and higher canopies, more shoot biomass and grain yield (for barley in Denmark), although in Sweden the amounts of biomass and grain for wheat were lower in the intercrops. These observed differences point towards a high complexity of trait plasticity, which is influenced by many different factors, including genotype-environment ($G \times E$) interactions. The increased tiller production of cereals in the intercrops may be attributed to higher N capture. In the northern latitudes where this study was carried out, the long days are known to negatively impact tiller production, considerably reducing cereal yield potential (Peltonen-Sainio, Jauhiainen, & Sadras, 2011), as shown for our wheat grown in Sweden compared to Denmark. These observations highlight some of the challenges with implementing intercropping in practice. For example, intercrop performance (e.g., grain yield, biomass yield) as compared to sole crop performance is in most cases context-specific (Brooker et al., 2015; Bybee-Finley & Ryan, 2018), implying the generalization of results should be done carefully. Such context specificity may result from the species or cultivar (with their respective functional trait values) as well as the environmental conditions.

Trait spaces of different species in sole crops and intercrops

While the mean values of the different functional traits each provided good insights on the performance of the different crop species when intercropped, the trait space analysis offered an even stronger basis for understanding species interaction. We found similar trends in functional trait spaces for crops in the pea-barley and faba bean-wheat species combinations, with larger hypervolumes in the intercrops than in the sole crops, except for wheat in Denmark. Based on the limiting similarity hypothesis, the low functional trait variation in the sole crops has potential in shaping plant-plant interactions by increasing intraspecific competition (Bennett et al., 2016; MacArthur & Levins, 1967). The consequence is that the low trait variability seen in the sole crops can negatively affect plant performance (e.g., in biomass production or grain yield) in those cases where a single

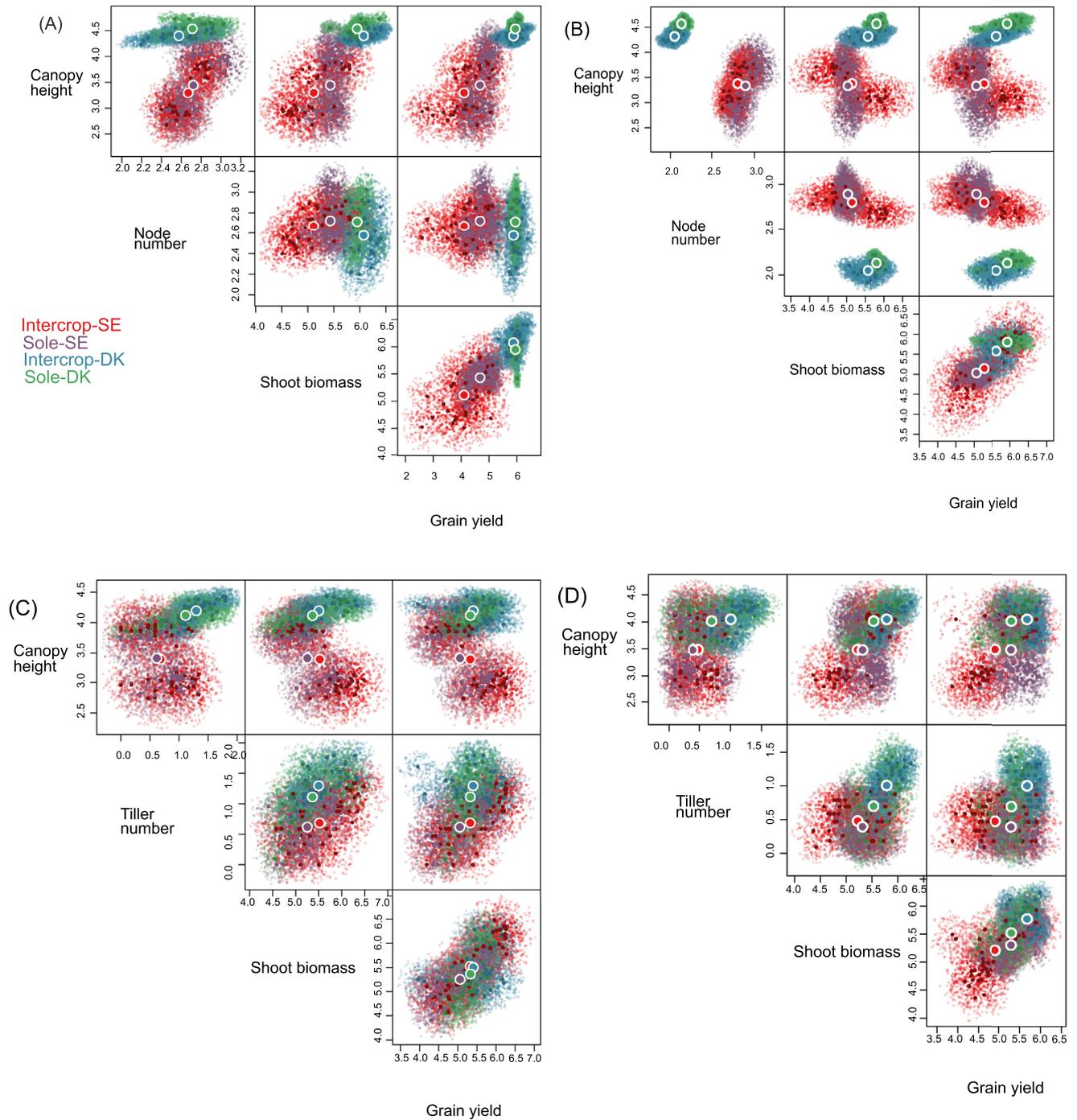


Fig. 2. Plots representing trait hypervolumes (units in SD4) for: (A) pea, (B) faba bean, (C) barley, and (D) wheat grown in Uppsala, Sweden (SE) and Copenhagen, Denmark (DK) as sole or intercrops. Based on Gaussian kernel density estimation of the measured values. Data were pooled for all cultivars of each crop species grown in 2017 and 2018. Large circles are hypervolume centroids, small darker points are data points, and small lighter nuances of each of the four colors are randomized points generated from the inferred hypervolume.

species is grown. Previous studies have shown that differences in hypervolumes may also be dependent on the identity of the cultivar used in the intercrop (Ajaj et al., 2021). Similarly, we found differences among cultivars for wheat, faba bean and barley in Sweden, and barley in Denmark. The observed differences in cultivars and whether species are grown in sole or intercrops may also depend on the choice

of the traits included in the analysis, as different traits may have a different relative contribution to the hypervolume and trait space. Hence, generalizing the results or applying them in another context should be done with caution. A starting point for ensuring the reliability of the concept is by assigning traits that are independent and uncorrelated as hypervolume axes. Although the differences in plant

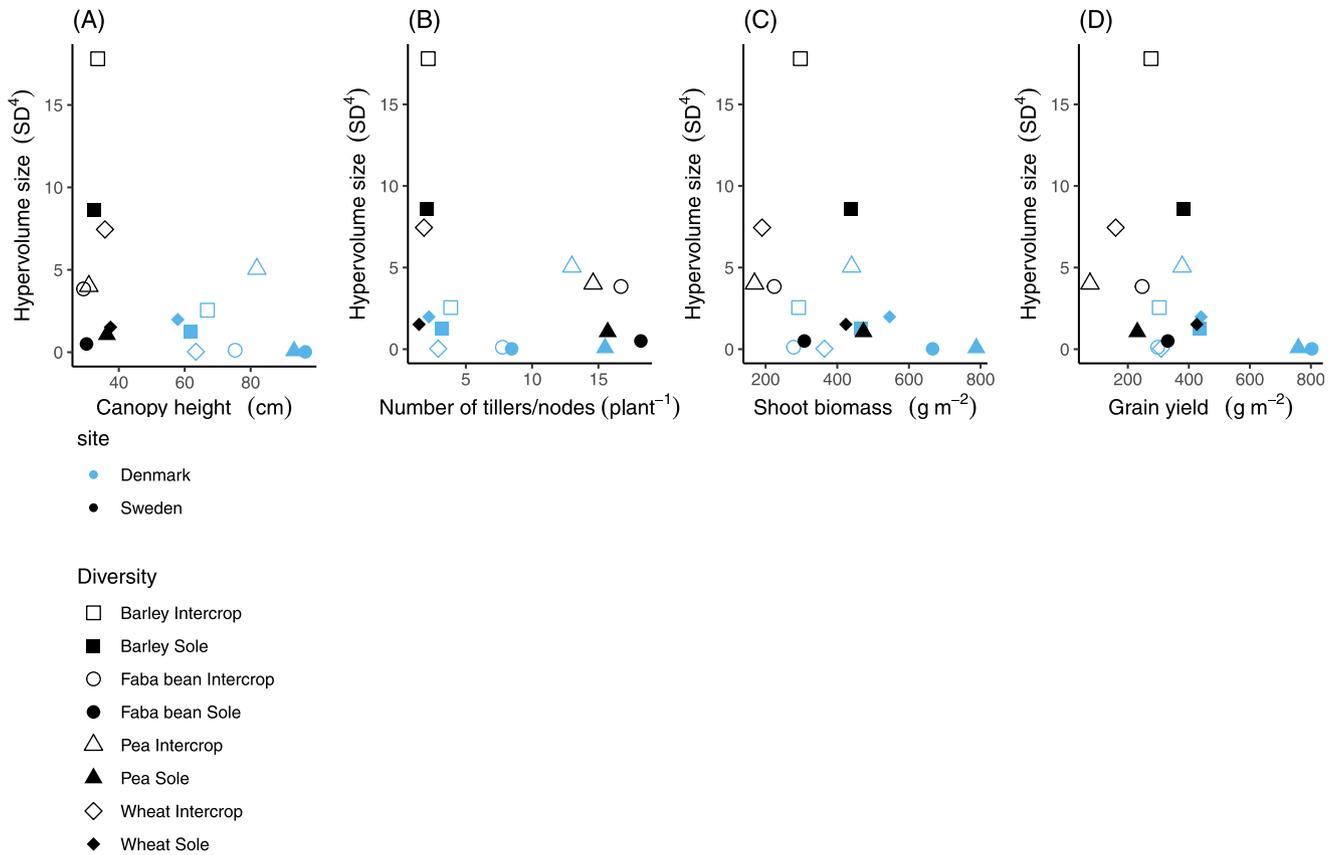


Fig. 3. Relationships between hypervolume size and trait value means of the four plant traits ((A)-(D)) assessed in pea, barley, faba bean and wheat grown at two sites as sole or intercrops. The points are means of each crop and trait values were averaged across cultivars and years. The closed and open symbols represent different crop species grown as sole and intercrops, respectively (see legend).

functional traits of mixture components were in most of our cases associated with increased niche complementarity and hence reduced interspecific competition, drawing resources from different niches may also result in the more competitive species outcompeting and dominating the less competitive ones as shown in the context of coexistence (Kraft, Godoy, & Levine, 2015). Large hypervolume size was associated with a large unique volume fraction and vice versa in some cases, as was also found in a few instances by Ajal et al. (2021). While the hypervolume size and unique volume fraction trend indicate large trait variation, this provides less information on the extent of niche sharing, specifically if no additional measures of overlap are used. Therefore, hypervolume size, together with other measures of overlap such as Euclidian centroid distance and Jaccard similarity should be used to minimize the unreliability of solely using intersection metrics (Mammola, 2019)

Effects of intercropping and growth conditions on trait plasticity

We have demonstrated that crop species when intercropped can facilitate a shift in the hypervolume dimensions. The

induced trait changes might have been accomplished through phenotypic plasticity similar to the cases of growing plants with different neighboring species or exposing them to other environmental cues that have been shown to drive phenotypic plasticity (Nicotra et al., 2010). We expected that the physiological mechanism controlling tiller formation in cereals would give barley and wheat a greater plastic ability when grown in sole or intercrop compared to the legume species under similar treatment. We found that the legume species also had significant plastic responses, and there was only one case in the cereal species (barley grown in Denmark) where intercropping had no influence on the plasticity of traits. It is challenging to pinpoint an explanation for this finding, as multiple drivers may act in concert to cause a phenotypic change. One confounding factor could be the high amount of soil N that likely caused this resource to not be a strongly growth-limiting factor in the two sites of our study (Jäck, Ajal, & Weih, 2021). Cereals are known to exhibit large plasticity of root traits under low-N conditions (Melino et al., 2015), which may affect overall plant growth (including tillering and shoot growth) to cope with the N-limitation. In such cases of N-limitation, the difference in phenotypic plasticity between cereal and legume species may be more apparent than in our study.

Crops grown in Denmark generally had high mean trait values irrespective of whether they were grown as sole or

intercrops, although they were associated with smaller trait space. Both sites are located at relatively high latitudes with favorable weather in terms of rainfall, and this was especially true in Denmark in 2017 (Weih et al., 2021), which may partly explain the higher trait values than in Sweden. Although the high mean trait values under more productive conditions in Denmark conform to our expectation, the small trait spaces were unexpected. For the traits we investigated, higher trait values are beneficial to the crop species, except for canopy height in the cereals, where higher trait values may imply taller plants that are prone to lodging. As a response to environmental conditions, plants exhibit adaptive plasticity to attain a competitive advantage over neighbors and modify their traits to better obtain limiting resources required for growth (Benavides, Valladares, Wirth, Müller, & Scherer-Lorenzen, 2019; Nicotra et al., 2010). In this study, we found that trait plasticity differed between sites when crops were grown in Denmark or Sweden. We cannot rule out that part of the site differences were caused by regional seed sourcing or the partly different cultivar combinations used in the Swedish and Danish trials.

The use of multiple traits in the trait space analysis highlighted the contribution of these traits to the performance of the different species in sole or intercrops, which offers a better understanding of the niche-based mechanisms for species' performance in intercrops. We used four functional traits that all relate to aboveground biomass accumulation. A core question that remains to be answered is whether the consideration of more or a different set of functional traits would result in similar findings and conclusions. Incorporating belowground traits or traits affecting multitrophic interactions (Brandmeier et al., 2021), is an aim for future studies that relate functional traits to crop performance in intercrops. Further exploring these questions holds promise for better understanding and implementation of breeding programs for mixed crop species.

Conclusions

Through the use of functional traits in cereal-legume intercrops grown under different environmental conditions, we have demonstrated that differences in trait space are influenced by intercropping and the cultivar used in the intercrop. Cultivar differences had a greater contribution to trait plasticity in Sweden than in Denmark, while management (fertilization level and pesticide application) influenced trait plasticity more in Denmark. Growing species as sole or intercrop had a significant effect on the expression of traits in both sites. Mean trait values and their correlations are still relevant indicators for quantifying the performance of intercrops as compared to sole crops, and the relationships between response and performance traits. However, analytical approaches based on trait space, accounting for multiple traits, provide a better understanding of the processes that generate the overall trait diversity observed in intercrops,

and the general mechanisms underlying the frequently observed yield advantages in intercrops; e.g., plasticity in our case. This study is relevant in elucidating the niche-based mechanisms underlying the interaction of species intercrops in an agricultural context.

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Declaration of Competing Interest

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

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Supplementary materials

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