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Functional trait space in cereals and legumes grown in pure and mixed cultures is influenced more by cultivar identity than crop mixing

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A B S T R A C T

More efficient resource use, especially nitrogen (N) in agricultural fields could considerably reduce the losses and spillover effects on the environment. Cereal-legume mixtures can lead to more efficient uptake of growth-limiting resources, and increase and stabilize yields, due to the variation in functional traits that facilitate partitioning of niche space. Here we identify crop mixtures with functional traits that facilitate optimal N resource use in two selected cereal-legume mixtures by using the multi-dimensional trait space concept. Combinations of pea-barley and faba bean-wheat crops were grown in the field as pure cultures and mixtures in Central Sweden, during two years with contrasting weather. The ecological niche space was defined via the *n*-dimensional hypervolumes represented by N pool, tiller/branch number, shoot biomass, and grain yield functional traits. Regressions and correlations allowed quantifying the relations between functional traits and plant N pools. Differences in trait space were not a result of crop mixing *per se*, as similar hypervolumes were found in the pure culture and mixture-grown crops. Instead, the trait space differences depended on the cultivar identities admixed. Furthermore, cereals increased their efficiency for N uptake and therefore benefitted more than the legumes in the mixtures, in terms of accumulated N and grain yields. Tiller and shoot biomass production in cereals was positively correlated to N pool accumulation during the season. Resource acquisition through increased N uptake in the mixture was associated with a reduced overlap in niche-space in the mixtures, and initial seed N pools significantly contributed to within-season N accumulation, shoot and tiller production.

1. Introduction

The use of cereal-legume crop mixtures can be associated with many benefits; for example, increased yield (Li et al., 2001; Ramirez-Garcia et al., 2015) and yield stability of the cereal-grain legume mixtures (Lauk and Lauk, 2008; Raseduzzaman and Jensen, 2017), improved grain quality and more efficient use of nutrient and water resources compared with the corresponding pure cultures (Li et al., 2006).

Specifically, more efficient resource use is one key attribute that practitioners of mixed cropping intend to achieve, through the exploration of different resource niches (Bedoussac et al., 2015; Brooker et al., 2015). Major growth-limiting resources are water, light, and nutrients. Nutrients often are more important than the other resources in northern-temperate regions, where water limitations for crop growth are infrequent and days are long during the growing season. Among nutrients, nitrogen (N) is focal when dealing with cereal-legume mixtures as legumes can symbiotically fix N_2 from the air, thereby considerably reducing the reliance on external fertilizer input (Jensen et al., 2020; Stomph et al., 2019). For example, a meta-analysis of yield gains in intercropping showed that mixtures can save up to 19–36 % of fertilizer in comparison to pure cultures grown under similar management (Li et al., 2020). Additionally, enhanced N uptake is found to occur in non-legume components when grown as mixtures with legumes (Duchene et al., 2017; Ramirez-Garcia et al., 2015), although in some cases this happens at the expense of the legume (Corre-Hellou et al., 2006; Duchene et al., 2017). The increased efficiency in resource use is attributed to the enhanced field level heterogeneity and positive interactions that could result from the mixing of different crop cultivars (intraspecific diversity) or two or more crop species (interspecific diversity) at the same time (Mansion-Vaquié et al., 2019). To mechanistically link the plant community responses and crop mixing to the properties of the involved species and cultivars, their functional traits can be studied.

According to Violle et al. (2007), plant functional traits refer to morpho-physio-phenological plant characteristics that indirectly affect plant fitness and influence individual performance in terms of growth,

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reproduction and survival. Functional traits are frequently discussed in relation to species differences, but variation in functional traits occurs indeed at both inter- and intra-specific levels where it can be affected by the environmental context and plasticity (Violle et al., 2012). In this study, we considered functional traits to be potentially expressed differentially depending on environmental factors (e.g., resource availability) or plant-plant interaction involving different species and cultivars. The environment or management (e.g., growing mixtures) can act as drivers for intra- or inter-specific variability. In a mixed crop system, different functional traits determine the crops' agronomic value (e.g., biomass or grain yield), while other traits important in plant-plant interaction define how one component in a species diverse crop system (here the mixed crop) affects the other and vice versa, e.g., rooting depth or the plant architecture (Litrico and Violle, 2015). Considering root functional traits to investigate below ground interactions and shoot traits for above ground interactions would be ideal. However, sampling root traits under field conditions, especially in mixed crop species, is challenging (Tosti and Thorup-Kristensen, 2010). Owing to this challenge, we focused on relevant and more accessible above ground traits, which also mirror aspects of plant resource use and are thus of relevance to be included in the framework that represents the various aspects of N accumulation and use efficiency. For instance, Shen et al. (2019) showed that certain above ground traits are good predictors for root traits, and an example relevant to this study is leaf nitrogen content predicting root nitrogen content. In crop mixtures, synergies that result from the functional trait differences in the mixture components must be maximally exploited, and negative interactions (e.g., competition) reduced. These needs drive breeding programs specifically targeted to crop mixtures, aiming at selecting cereal-legume components leading to high resource use, thanks to the interaction of their functional traits (Benavides et al., 2019). More recent approaches to quantify species interaction in mixtures take into account the functional traits of different species, rather than the species' performances themselves (de la Riva et al., 2017), particularly through characterizing the contribution of traits to exploit resources from a common niche. The functional trait space concept, first proposed by Hutchinson (1957), is based on *n*-dimensional hypervolumes, where *n* represents the number of traits under investigation or limiting resources required by individual plants in a given environment (Blonder, 2018; Blonder et al., 2014). The trait space concept allows quantifying niche overlap in diverse plant communities as opposed to pure communities consisting of only one species. It has been effectively used in natural grasslands, and it holds promise also for cultivated crops species, and specifically cereal and legume crops grown in pure and mixed (i.e., more diverse) culture. The trait space approach allows the separation of different resource capture or utilization strategies with respect to the components in a mixture through niche partitioning (de la Riva et al., 2017).

In this study, we applied a multi-dimensional trait space approach to evaluate N use and N pool dynamics in pea-barley and faba bean-wheat plant teams grown in Central Sweden (Fig. 1). Based on a number of selected above ground functional traits, which represent the various aspects of N accumulation and use efficiency (Weih et al., 2011, 2018), we intended to find cultivars with trait combinations that promote more efficient N resource use in the species mixtures. Specifically, we hypothesized that:

(H1) Depending on crop mixing (i.e., pure or mixed culture), different cultivars of each species display different trait space values and hypervolume overlap, with the mixtures having larger volume sizes than that of the same species grown in pure cultures.

The yield advantage in mixed cultures is partly associated with the

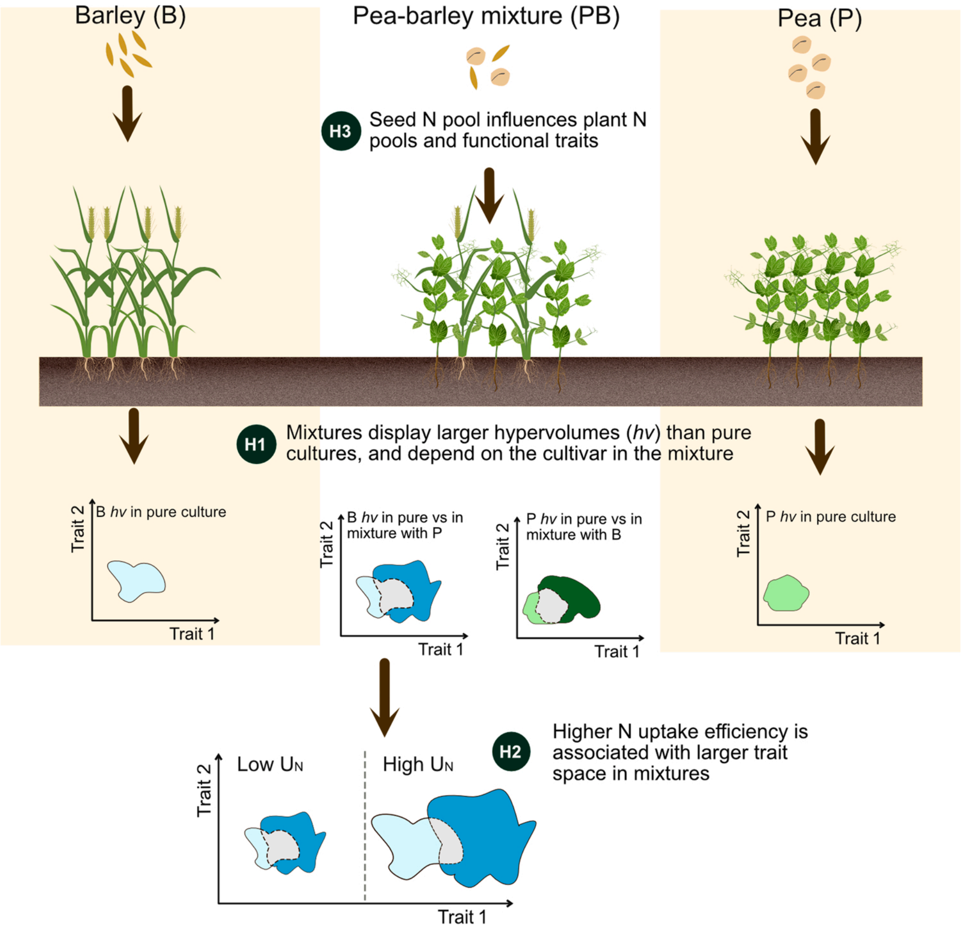

Fig. 1. Conceptual diagram illustrating the connection among the hypotheses (*H1, H2* and *H3*) with the example barley grown in pure culture and in mixture with pea. Hypervolumes in pure and mixed cultures are represented in light and dark colours respectively. The hypervolumes for pure culture is included in *H2* for reference. The initial seed N pool facilitates early seedling vigour, which later, during the crop growth period, contributes to the expression of different plant functional traits such as tiller development, biomass and N pool accumulation. The trait differences between mixture components are the drivers of weaker resource competition in mixtures arising from complementary and facilitative mechanisms associated with larger trait space and increased N uptake efficiency (U_N) in the cereal component.

different functional traits values of the mixture components that facilitate more efficient resource use. Most important is whether the crop system is superior in resource acquisition or conversion of the acquired resources into yield or products (Stomph et al., 2019). Hence, we further hypothesize that *(H2)* Larger functional trait spaces (with minimal overlap) are associated with increased N uptake efficiency (improved N acquisition) in the mixtures rather than enhanced grain specific N efficiency (N conversion efficiency) over the growth period; and *(H3)* Initial seed N pool influences above ground functional traits through N accumulation, tiller, and shoot biomass production.

2. Materials and methods

2.1. Field site

Field experiments were established at approximately 500 m apart in the 2017 and 2018 growing seasons at the Säby field station, Uppsala, Central Sweden (59°49′54.3″ N 17°42′07.9″ E). Soils were composed mainly of silty clay with 5.1 and 3.2 % organic matter content in 2017 and 2018 field sites respectively. The top 0.3 m of the soil had mineral contents of 39 kg N ha^{-1} and 78 kg N ha^{-1} prior to fertilization in 2017 and 2018, respectively, which seemed sufficiently available and non-limiting for plant growth. The region is characterized by a cool temperate climate under which spring-sown crops favorably grow between May and September. Average mean monthly temperatures during the 2017 growing season ranged between 10 °C–16 °C while the 2018 growing season was warmer with average temperatures between 13 °C–21 °C, and drier compared with the historical average of the same period (Table 1). Due to the dry period experienced in 2018, emergency irrigation of 10 mm of water was applied at 39 and 44 days after sowing (DAS), which coincided with tillering and stem elongation stages in the cereals and legumes respectively.

2.2. Plant material and experimental design

The plant material comprised of cereals; spring wheat (*Triticum aestivum* L.) and spring barley (*Hordeum vulgare* L.), legumes; pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.); all grown separately and as barley-pea and wheat-faba bean species combinations. Within each species combination, one cultivar of each species was grown as pure culture, and as two-species mixture. In total, three cultivars of each cereal, i.e., barley (cv. 'RGT Planet', 'Tamtam' and 'Vilgott') and wheat (cv. 'Diskett', 'KWS Alderon' and 'Cornetto') were grown; and two cultivars of each legume, i.e., pea (cv. 'Ingrid' and 'Clara') and faba bean (cv. 'Fuego' and 'Boxer') were used, resulting in the following 12 combinations or plant teams: RGT Planet-Ingrid, RGT Planet-Clara, Tamtam-Ingrid, Tamtam-Clara, Vilgott-Ingrid, Vilgott-Clara (barley-pea), and Diskett-Fuego, Diskett-Boxer, KWS Alderon-Fuego, KWS Alderon-Boxer, Cornetto-Fuego, Cornetto-Boxer (wheat-faba bean). 'KWS Alderon' and 'RGT Planet' cultivars are hereafter referred to as 'Alderon' and 'Planet' respectively. The results presented for each crop included each of the three cereal cultivars grown in pure culture and with one legume cultivar (faba bean cv. 'Fuego'; pea cv. 'Ingrid'), and each of the two legume cultivars grown in pure culture and in mixture with one cereal cultivar (wheat cv. 'Diskett'; barley cv. 'Planet'). The distinction between the one-species and two-species mixtures was used as a measure of the mixing effect and referred throughout this paper as crop mixing level. The barley-pea and wheat-faba bean species mixtures and cultivars were selected based on local availability and farmer preferences. Seeds were sown on May 5th in 2017, and April 30th in 2018 at a depth of 50−60 mm. The sowing rates for pea, faba bean, barley and wheat were 90, 60, 400, 490 seeds m^{-2}, respectively, in the pure culture, with the mixtures of each species having half the proportion of seed amount in pure culture laid in a replacement design. Mixing was done within each row measuring 12.5 cm apart in plots of 10 m × 2 m in size. At 26 DAS, fertilizer treatment with 90, 15 and 29 kg ha $^{-1}$ of N, P and K, respectively, was applied to the barley and pea; and 140, 24 and 46 kg ha^{-1} of N, P and K, respectively, were applied to the wheat and faba bean. Control treatments with no additional N were included for all pure and mixed cultures. The plots were arranged in a split-plot design with fertilizer treatment as the main plot and cultivar/species mixtures as the sub-plots in four replicates. Pest and disease infestation did not occur in the experiment or only at negligible levels, and no active weed management was done.

2.3. Field sampling

Destructive samplings of vegetative biomass were done at two time points, at crop flowering (59 DAS in 2017; 65 DAS in 2018) and at physiological maturity (123 DAS in 2017; 107 DAS in 2018) of the cereals as these mark major physiological stages during a crop life cycle. Biomass samplings and measurements were done as follows; samples were taken by harvesting 0.5 m^2 from each plot (two times using 0.25 m^2 quadrats) at flowering and at maturity. The number of tillers (cereals) and shoots (legumes) per m^2 were counted. The grain dry matter yield assessment at maturity was done by harvesting the central 12 m^2 of each plot using a combine harvester. The grain yield per plot was calculated by multiplying the grain yield in the sub-sample by 100 % in the pure culture, and by the percentage proportion of each species in the mixture that made up the total grain yield in the sub-sample. In addition to the plot-level data, five representative plants per species were taken at both sampling occasions from each plot to record tiller number in cereals, branch number in legumes, pod/head weight, thousand kernel weight, and crop biomass for N analysis. The number of tillers per plant and the total number of counted tillers per m^2 were used to estimate the number of cereal plants per m^2. All samples were packed in separate bags according to species and dried at 70 °C for 48 h. Yield evaluations were done both at plot and individual plant levels with the two methods showing significant positive correlation ($r = 0.68$ and $p \leq 0.001$; Fig S1). The grain yields obtained were used in the calculation of Land Equivalent Ratio, LER (Mead and Willey, 1980). The LER value greater than 1 indicate a yield advantage of mixtures compared to the pure cultures.

2.4. Nitrogen analysis and accumulation efficiency calculations

To obtain total plant N in shoots and grains, samples were ground

Table 1
Mean monthly temperature and precipitation for the 2017 and 2018 growing seasons and long-term averages over the period 1950-2019. The days that received precipitation during the growing season in 2017 and 2018 are also included.

Period	Mean temperature °C			Precipitation sum (mm)			Days with precipitation >1 mm (days)	
	2017	2018	1950−2019	2017	2018	1950−2019	2017	2018
May	10.4	15.3	10.3	10.4	6.7	34.8	2	2
June	14.7	16.3	14.8	50.6	20.7	52.0	10	3
July	16.6	21.6	16.9	14.6	81.7	64.1	8	2
August	15.8	17.8	15.7	61.1	68.7	68.6	8	11
September	12.2	12.9	11.2	68.2	42.2	51.0	7	12

Source: Ultuna climate Station, Uppsala (Sweden) located a few km from the field site.

into a fine powder and 0.5 g obtained as a subsample and analyzed after dry combustion (Dumas principle, ISO 13878) using a LECO CN-2000 analyzer.

The N accumulation efficiency, NAE, and its components (Weih et al., 2011, 2018) were calculated to link N resource uptake and utilization to productivity. Mean N pools over the growth period were calculated from plant N contents (total plant N) in the shoot (leaves, stems, and seeds) of each species grown in pure or mixed culture. Seed N pool, which accounts for the N carry over from the previous season, was derived from the N contents of the planted seeds. The NAE (g g^{-1}) can be decomposed in three components:

$$NAE = U_N \times E_{N.g} \times C_{N.g} \tag{1}$$

where

U_N (g g^{-1})Mean N uptake efficiency during the major growth period. It is calculated as the ratio between the mean plant N content during the entire growing season (N', g m^{-2}) and the initial N content in the grain seeds (N_s, g m^{-2});

$E_{N.g}$ (g g^{-1})Grain specific N efficiency, which is the ratio between the grain biomass produced at final harvest (B_g, g m^{-2}) and N';

$C_{N.g}$ (g g^{-1})Grain N concentration at final harvest, which is the N content of produced grain at final harvest N_g (g m^{-2}), divided by B_g.

2.5. Hypervolume construction and trait space analysis

The hypervolumes for trait space analysis were constructed using the Gaussian kernel density estimation method (Blonder et al., 2014, 2018). The analyses were implemented in the ‘hypervolume’ R package (Blonder and Harris, 2018). Four traits – grain yield, nitrogen pool, tiller/branch number, and shoot biomass – were included in the hypervolume (trait axes) because of their relevance to N uptake and utilization in plants. Before the analyses, all measured data were standardized by z-transformation to enable comparison of trait axes with different units. The measured data set only allowed the construction of a single hypervolume for each combination of traits in focus. Therefore, we did repeated simulations using the replicate() function in R (Muldoon, 2018) to generate 10 replicates of random variables drawn from a normal distribution, with mean and standard deviation of the measured data for the corresponding hypervolumes. This facilitated quantification of the variability in the hypervolumes and testing for statistical significance difference of trait space parameters between treatments. Hypervolumes were then constructed for each species component grown at the different crop mixing levels.

The 4-dimension trait hypervolumes (SD^4) were explored visually based on only measured data and using all cultivars of each species, but further analyses presented below were performed for three traits using measured and simulated data. The grain yield was excluded because the focus of the analysis was on above ground functional traits that influence N utilization with the goal of increasing yield, and not on yield itself. The 3-dimension hypervolumes (SD^3) were created separately at species level for each cultivar grown at the different crop mixing levels. All legume cultivars were tested when grown with the same cereal cultivars, and similarly, all cereal cultivars grown with the same legume partner were tested.

Four metrics describing the hypervolume properties were considered: the hypervolume size, niche overlap evaluated by the Jaccard similarity and unique volume fraction, and centroid distance. Jaccard similarity denotes the fraction of the combination of two hypervolumes A and B occupied by the intersection of the two:

$$\text{Jaccard similarity} = \frac{A \bigcap B}{A \bigcup B} \tag{2}$$

where $A \bigcap B$ is the volume of intersection A and B, and $A \bigcup B$ is volume of the union of A and B. Similarly, for hypervolume A, the unique volume fraction is the volume of unique component of A (i.e. not in common with B) divided by volume of A. The centroid distance determines the distance between the centers of two hypervolumes.

2.6. Statistical analysis

All statistical analyses were performed in R version 3.6.1 (R Core Team, 2019). Each cereal and legume partner was analyzed separately for all the attributes investigated and all differences reported were significant at $\alpha = 0.05$. Tests for assumptions of normal distribution and equal variances were done either visually or using Levene’s test for equality of variance. Non-normally distributed data were log-transformed (log 10) before analysis. Trait space parameters evaluated at each crop mixing level were analyzed through a two-way ANOVA to determine the effect of Mixing level and Cultivar, and for trait space parameters evaluated using data from both crop mixing levels, the effect of Cultivar was analyzed through a one-way ANOVA. All the other parameters were analyzed through a linear mixed effect model [nlme Package: ‘*lme*’ by Pinheiro et al. (2017)] and Tukey’s pairwise comparison of means using emmeans (Lenth, 2019). Model structure depended on the parameter. For NAE components, factors N-level, Mixing level, Cultivar, and their interaction were considered as fixed effects, and N-level nested within blocks as random effects. For grain yield, each cultivar team was analyzed separately with N-level, Mixing level, Year, and their interaction treated as fixed effects, and N-level nested within blocks as random effects. Additionally, linear regression for the relationships between total N pools and tiller number, and shoot biomass of barley and wheat were performed.

3. Results

3.1. Trait space is influenced more by functional trait differences among the cultivars than by crop mixing

Plots representing 4-dimensional hypervolumes were used to visualize hypervolumes at the different crop mixing levels (i.e., pure and mixed culture), based on randomly sampled points from the inferred hypervolumes (Fig. 2). Most crops had similar hypervolume sizes at the different crop mixing levels, except faba bean that had larger hypervolumes in pure culture than in the mixture ($p = 0.048$; Table 2). However, there was an effect of cultivar identity on hypervolume size in all crop species ($p \leq 0.001$ in both barley and wheat, faba bean; $p = 0.008$, and for pea; $p = 0.025$). Additionally, we found a strong cultivar-mixing effect in faba bean ($p \leq 0.001$) and barley ($p \leq 0.001$), with only a marginal effect observed in wheat ($p = 0.050$). In wheat, ‘Alderon’ had larger hypervolumes than ‘Cornetto’ (Table S1). In barley, hypervolume differences resulted mainly from large hypervolumes in mixture-grown ‘Planet’ and pure culture-grown ‘Vilgott’ in comparison to the other cultivars (Table S2). Hypervolume size differences in faba bean were a result of ‘Boxer’ grown as pure culture, and ‘Fuego’, irrespective of the crop mixing level having larger volumes than ‘Boxer’ in the mixture.

The unique volume fraction followed a similar pattern as in the hypervolume size in the cereals but not in the legumes. In the cereals, different cultivars of wheat and barley varied in their unique volume fractions (Table 2; Fig. 3I). In addition, cultivar identity at different crop mixing levels led to significant cultivar-mixing level effects ($p = 0.027$ in wheat and $p < 0.001$ in barley). However, in faba bean, the unique volume fraction differed between the two crop mixing levels depending on the cultivar, although the cultivar identity in itself had no effect.

The Jaccard similarity indices were different among the cereal cultivars (wheat; p=0.008, barley; p= 0.002). Among the legumes, differences were only observed in faba bean (Table 2; Fig. 3 II d and h). ‘Diskett’ and ‘Alderon’ wheat cultivars had on average lower similarity in volumes than ‘Cornetto’. In barley, the highest similarity of 0.38 was observed in ‘Vilgott’, which was larger than the values obtained in ‘Planet’ ($p = 0.003$) and ‘Tamtam’ ($p = 0.011$). Consequently, ‘Planet’ and ‘Tamtam’ barley cultivars had larger centroid distance than ‘Vilgott’

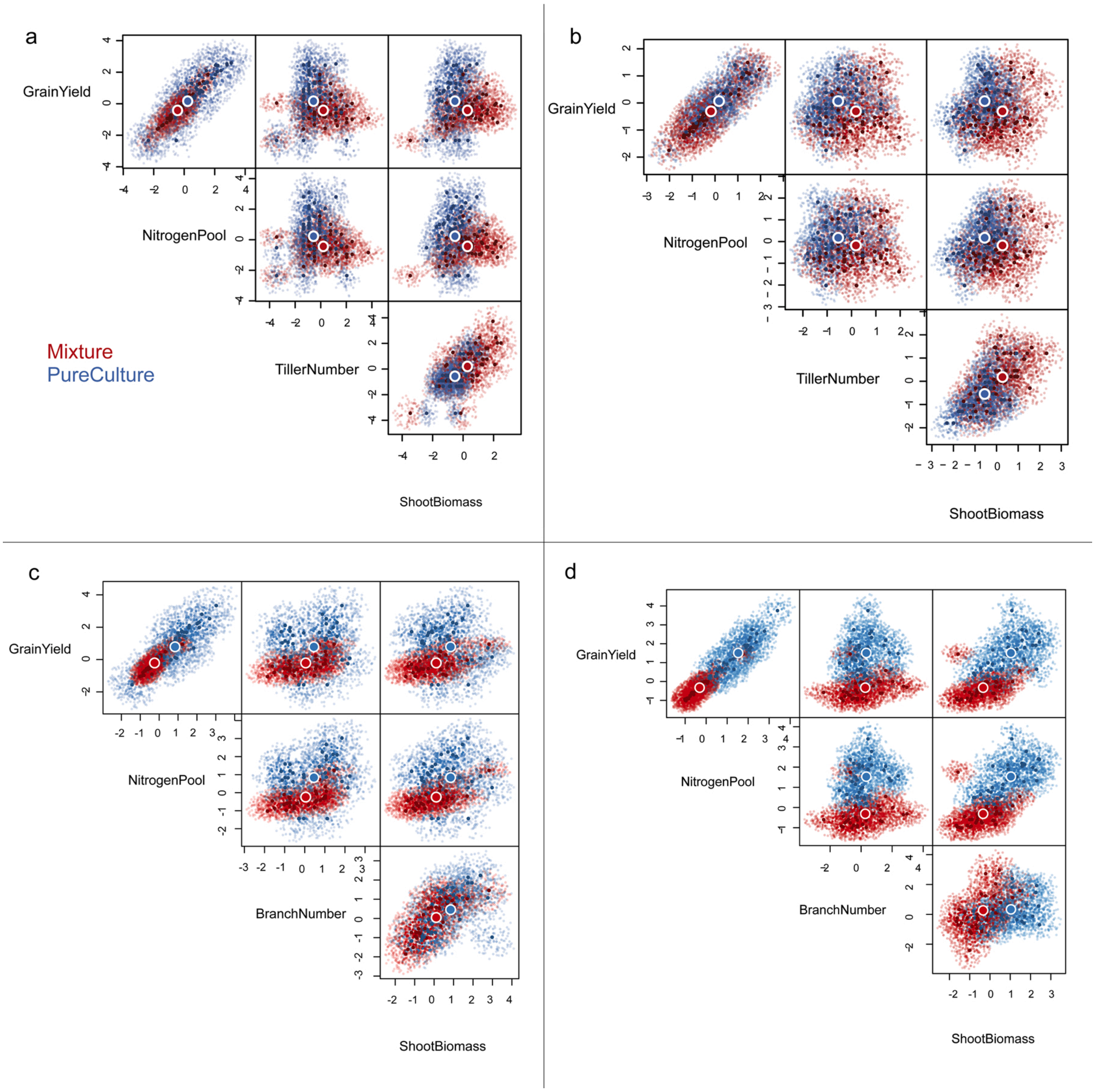

Fig. 2. Plots representing hypervolumes (SD^4) for wheat, barley, pea and faba bean traits based on Gaussian kernel density estimation. Hypervolumes for (a) three wheat cultivars grown in pure culture and in mixture with faba bean cv. 'Fuego', (b) three barley cultivars grown in pure culture and in mixture with pea cv. 'Ingrid', (c) faba bean cv. 'Fuego' and 'Boxer' grown in pure culture and mixture with wheat cv. 'Diskett', and (d) pea cv. 'Ingrid' and Clara grown in pure culture and in mixture with barley cv. 'Planet', using measured values pooled for all cultivars at each crop mixing level. The large points are hypervolume centroids, small dark circles are data points, and the light points represent randomized values generated from the inferred hypervolume.

(1.47; p=0.015 and 1.54; p=0.038 for 'Planet' and 'Tamtam', respectively), reflecting a larger functional trait space. In the legumes, pea cultivars had similar centroid distances and Jaccard similarity indices at the two crop mixing levels (Fig. 3 II c,d,g, and h), despite having the largest centroid distance (greater than 2 SD). The reverse was true for faba bean, which had smaller centroid distance although different among the cultivars.

3.2. *Larger functional trait space in the cereal-legume mixtures is associated with increased N uptake in the cereal partners*

Both wheat and barley grown with a legume partner had a higher N uptake efficiency, U_N in the mixture than in pure culture in both years (Fig. 4; Table S3 and S4). This consequently increased N resource use efficiency characterized by higher NAE values in mixtures than in pure cultures specifically in 2017. At community level, 'Boxer'-'Diskett' (faba bean-wheat) and 'Ingrid'-'Vilgott' (pea-barley) plant teams had higher U_N than all the other plant teams in 2017 and 2018 respectively; however, only 'Ingrid'-'Vilgott' resulted in different NAE (Table 3). The yield pattern at harvest (B_g) was contrary to the trends observed in U_N as both wheat and barley had higher $E_{N,g}$ in pure cultures compared to the corresponding cultivars grown in mixtures. There was an effect of cultivar on grain N concentration, $C_{N,g}$, whereas the crop mixing level mostly had no effect on this trait except for barley in 2017.

The relationship between multidimensional trait spaces and NAE for the different cultivars showed that small differences in trait space at the different crop mixing levels resulted in large differences in NAE for both the cereal and legume partners (Fig. 5). At the same time, there was a positive relationship between U_N and NAE in all species components (Fig. 6). Therefore, the high U_N of some cultivars contributed greatly to the high efficiency in multiplying the seed N to harvested N (i.e., high NAE; eq. 1) of the same cultivars. While for the cereals, the trait space was larger and NAE was higher in the mixtures than pure culture, the reverse was true in the legumes. In wheat, 'Diskett' and 'Alderon' cultivars grown in mixture had the highest NAE of about 53 g g^{-1} and with large volume sizes relative to all other wheat cultivars except 'Alderon'

Table 2
Analysis of Variance table with *F* and *P* values for hypervolume size, unique fraction (%), Centroid distance and Jaccard similarity for cereal and legume plant teams. Unique volume fraction was calculated from unique volume in intersection divided by the total volume of a given variable. Differences in Centroid distance and Jaccard similarity were analysed the different cultivars grown in pure culture and in mixture. Symbols show results with significant levels;*** $= P \leq 0.001$; ** $= P \leq 0.01$; * $= P \leq 0.05$; ns = non-significant.

Factor	Hypervolume		Unique fraction		Centroid distance		Jaccard simmilarity	
	F value	P value	F value	P value	F value	P value	F value	P value
Cereals								
Wheat and faba bean cv. Fuego								
Cultivar	59.750	≤ 0.001***	3.248	0.047 *	1.817	ns	5.637	0.008 **
Mixing level	0.001	0.982	0.441	0.509				
Cultivar × Mixing level	3.152	0.050	3.862	0.027 *				
Barley and pea cv. Ingrid								
Cultivar	7.286	0.001 **	6.786	0.002 **	5.281	0.011 *	8.143	0.002**
Mixing level	0.113	0.738	0.294	0.589				
Cultivar × Mixing level	24.914	≤ 0.001***	24.073	≤ 0.001***				
Legumes								
Faba bean and wheat cv. Diskette								
Cultivar	7.713	0.008**	1.406	0.243	2.376	ns	7.925	0.011*
Mixing level	4.153	0.048*	15.829	≤ 0.001***				
Cultivar × Mixing level	14.444	≤ 0.001***	40.648	≤ 0.001***				
Pea with barley cv. Planet								
Cultivar	5.410	0.025*	0.083	0.775	0.019	ns	0.036	ns
Mixing level	1.826	0.181	2.085	0.157				
Cultivar × Mixing level	0.062	0.805	0.012	0.912				

in pure culture. In light of this, admixed 'Diskett' specifically exhibited consistently larger unique volume fraction than in pure culture, had low Jaccard similarity value (0.24), and maintained a large centroid distance of 2.03 SD between the volumes at the different crop mixing levels, illustrating lower volume overlap. In barley, the mixtures had higher NAE but with relatively smaller volumes compared to the wheat. At the same time, the hypervolumes were more similar with relatively large Jaccard similarity values compared to wheat. Consequently, there were smaller distances between the centroids of different cultivars grown at the different crop mixing levels. In contrast to the cereals, the legumes had higher NAE in pure cultures than in the mixtures but the relationship with trait space varied. In pea, similar trait space parameters were observed despite higher NAE in the pure culture compared to the mixtures. At community level, high yields were achieved when 'Alderon' was mixed with any of the faba bean cultivars. 'Ingrid'-'Vilgott' also had high U_N and NAE in the 2018 growing season, while the larger hypervolume and unique volume fraction of 'Planet' in the mixture resulted in high community level $C_{N.g}$ in the 'Clara'-'Planet' plant team.

3.3. Initial nitrogen pools increased tiller production and biomass accumulation

The soil N level had no effect on functional traits as the grain yield and NAE components were similar in the treatments with and without additional N (Table S3 and S4). However, we found a positive correlation between seed N pool and tiller number in both wheat and barley in the two consecutive years (Fig. 7a and d). Similarly, increased seed N pool was associated with higher shoot dry weight in both crops except for barley in 2018 (Fig. 7 b and e). Consequently, both barley and wheat increased the total plant N pool accumulated in the biomass, specifically due to increased tiller number, except in 2017 where we noted no effect of tiller number on accumulated total plant N pool (Fig. 7 c and f). In general, a large total N pool was accumulated by wheat in the 2017 growing season despite the few tillers produced. For example at 200 tillers per m^{-2}, up to 20 g m^{-2} of plant N was already accumulated in wheat at crop flowering.

For the relationship between NAE or its components and shoot traits, we found the mean plant N content, N', to be positively correlated with tiller number, and shoot dry weight in wheat and barley in all cases (Table 4.) This implies the increase in mean N pool over the season was associated with more tillers and shoot biomass produced. Overall, there was no correlation between NAE and tiller number for both wheat and barley although high NAE was associated with increased shoot dry weight in both crops in the 2018 growing season (wheat; $r = 0.24$, $p = 0.045$, $n = 72$ and barley $r = 0.32$, $p = 0.006$, $n = 72$). In contrast, a negative correlation between NAE and tiller number was observed in wheat grown in 2017 ($r = -0.29$, $p = 0.0016$, $n = 72$). In addition, the NAE components U_N, $E_{N.g}$ and $C_{N.g}$ partly varied between crops and years. For example, the uptake efficiency, U_N, was negatively and positively correlated to tiller number in wheat ($r = -0.46$, $p = 0.001$, $n = 72$), and barley ($r = 0.26$, $p = 0.028$, $n = 72$), respectively, with similar trends followed with respect to shoot dry weight. A consistent negative correlation was observed between $C_{N.g}$ and tiller number in barley. For wheat, increased $E_{N.g}$ was associated with more tillers only in the 2017 growing season.

4. Discussion

This study is first in applying the niche-based concept of trait space for evaluating N resource use in managed agricultural plant communities, or crops; previous studies have applied this concept only to plants grown in their natural plant communities. We use trait hypervolumes to measure the overlap of ecological niche spaces in plant phenotypes (Blonder, 2018; Diaz et al., 2016) in pea-barley and faba bean grown at different crop mixing levels, to contrast species and cultivars differing in their N use. We demonstrated the relevance of this tool to quantify the effect of crop mixing, specifically in cereal-legume mixtures, highlighting the major drivers for the observed trends in trait spaces, as well as the mechanistic underpinnings of N resource acquisition and use efficiency. These were quantified based on trait values simulated from measured data, but the simulated and measured data followed a similar pattern (Table S5 and S5), indicating a good accuracy and reliability of the findings.

4.1. Trait space relations are influenced more by cultivar identity than the crop mixing levels

The need to devise management solutions for more efficient use of resources, such as N, in crop production necessitates quantifying the contribution of functional traits interacting with environmental factors or management actions (e.g., pure culture vs. mixture, or the choice of cultivars) to attain the desired yield. Distinguishing the cultivar effects

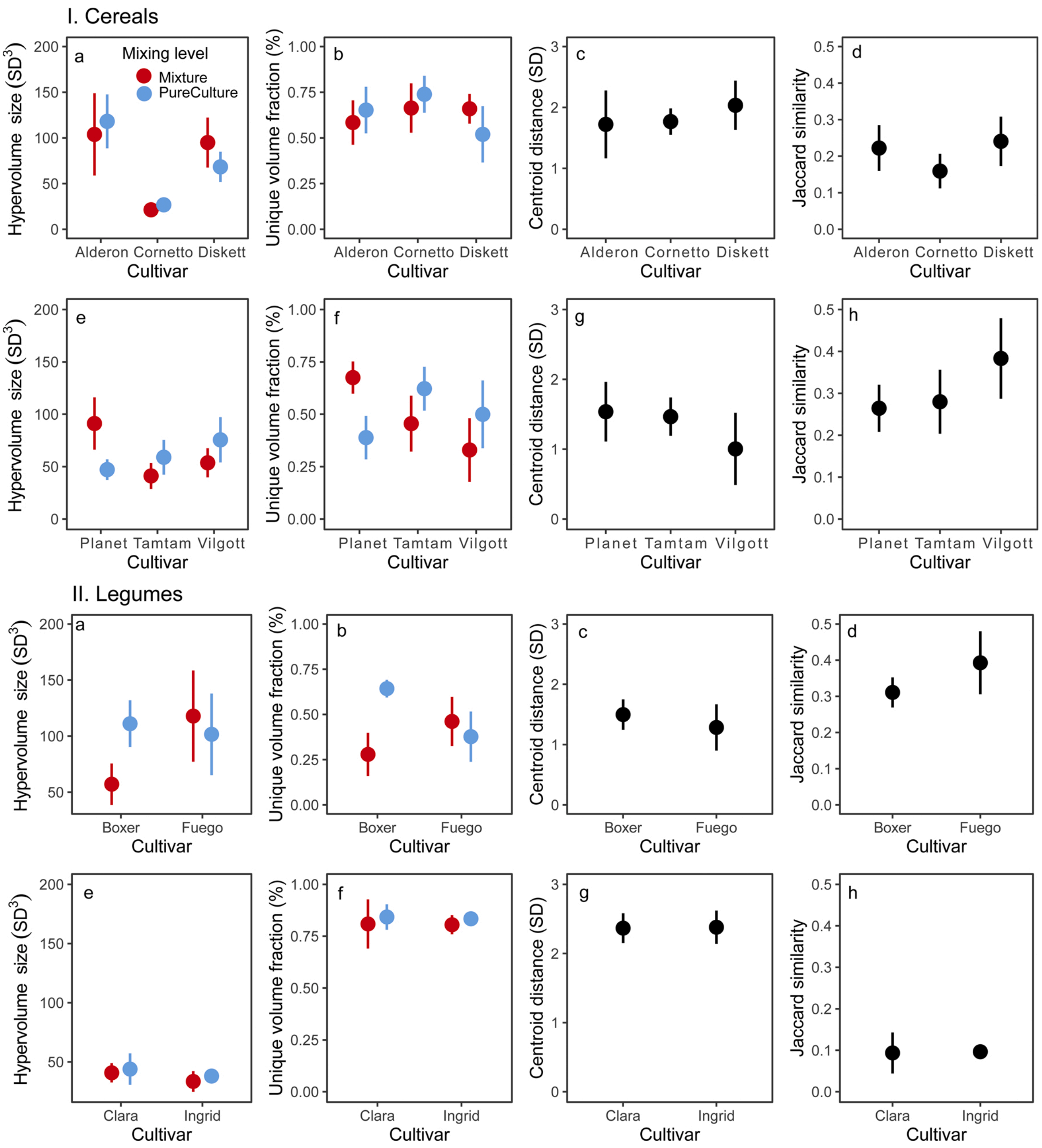

Fig. 3. Trait space analysis for wheat (I, a-d) and barley (I, e-h) cultivars grown at different crop mixing levels with a legume component. Faba bean cv. 'Fuego' and pea cv. 'Ingrid' were used for wheat and barley, respectively. Graphs II (a-d) show the performance of pea cv. 'Ingrid' and 'Clara' when grown at different crop mixing levels with barley cv. 'Planet', and II (e-h) faba bean cv. 'Fuego' and 'Boxer' with wheat cv. 'Diskett'. The error bars represent mean ± SD.

and mixture effects would facilitate better understanding of the major drivers and underlying mechanisms.

Our results indicate that species components grown at different levels of crop mixing had similar hypervolume sizes but differed based on the choice of the cultivar of each species, with the exception of faba bean and some barley cultivars. The cultivar differences also dominated when considering the unique volume fraction and Jaccard similarity, with centroid distance varying only among barley cultivars. These findings in part support *H1*, as different cultivars indeed differed in the level of overlap of trait spaces - specifically the cereals 'Planet', 'Alderon' and 'Diskett' cultivars displayed larger volume sizes in mixture than other cultivars. The major driver of trait space in the cultivars and traits we tested was the dissimilarity in functional traits, and to a smaller extent, trait plasticity of the cultivars and species components in the mixture. When we looked at the mixture effects, the legumes mainly differed from our expectation of larger hypervolumes in mixtures than the pure culture, which is typical in trait convergence where species show trait similarity to minimize competitive pressure (interspecific competition) for resources from another species (Grime, 2006). From a mechanistic perspective, the more diverse crop systems (here mixtures as opposed to pure cultures) promote complementary use of resources and facilitation (Hauggaard-Nielsen et al., 2009; Vandermeer, 1989), which arise from niche partitioning of resources, such as N.

Hypervolume size indicates the variability in phenotypes (or traits) under different growth conditions, suggesting that there was likely a phenotype change (or plasticity) in faba bean, and some barley cultivars depending on whether they were grown in pure culture or mixture. Indeed, plasticity also played a significant role in realized benefits in cultivar mixtures of barley (Dahlin et al., 2019). In addition, the yield advantage in mixtures stems from several facilitative or complementary

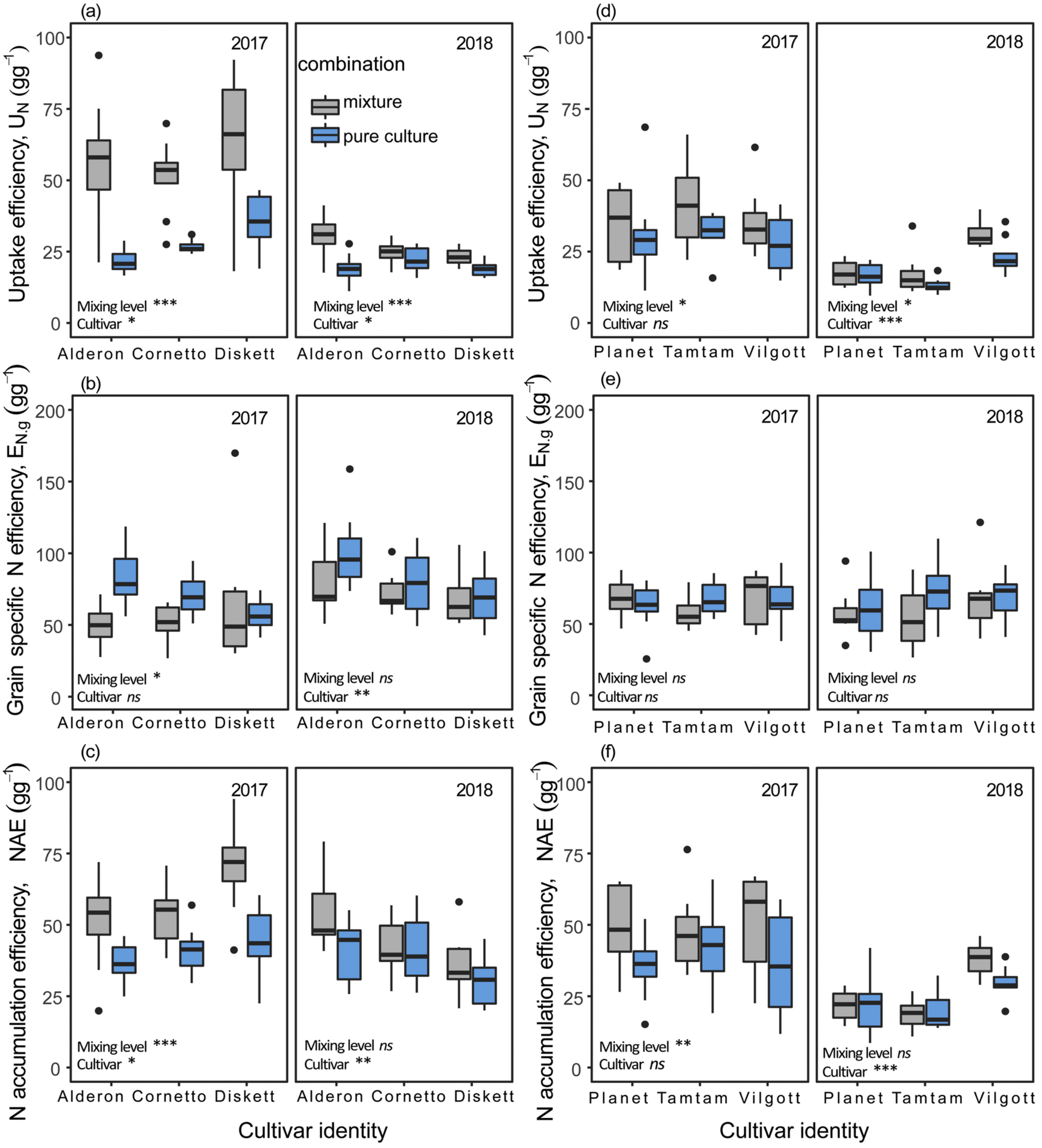

Fig. 4. Resource accumulation efficiencies for three different wheat cultivars grown as pure culture and in mixture with faba bean cv. 'Fuego'(a-c) and for three different barley cultivars grown as pure culture and in mixture with pea cv. 'Ingrid'(d-f). The analysis is based on five individual plants per species sampled per plot for years 2017 and 2018. Symbols show ANOVA results with significant levels; *** = $P \leq 0.001$; ** = $P \leq 0.01$; * = $P \leq 0.05$; ns = non-significant.

mechanisms, also beyond the above ground phenotypic traits assessed here. For example, a mixture of plants with diverse root architecture may enhance complementary resource capture (Brooker et al., 2015) or direct N transfer from legumes to non-legumes through root exudates or by arbuscular mycorrhizal fungi (Stern, 1993; Thilakarathna et al., 2016). We were unable to detect these mechanisms because the species-specific assessment of root traits in the field is extremely challenging especially in intercrops, with a high risk of generating unreliable data, and was not done in this study.

The trait space-crop mixing relations presented here suggest that achieving the benefits of greater diversity in crop mixtures is possible, although not always. But the cultivar choice can have significant influence on the realized benefits of mixtures. In this way, specific mixtures of crops (or cultivars) can be selected based on their functional traits to achieve the desired goal, for example for more efficient N resource use as shown in our study.

4.2. Larger functional trait space is associated more with increased N uptake efficiency than conversion efficiency

The emerging relation between resource acquisition and conversion with trait space in mixtures is one example illustrating how plants can take advantage of the differential expression of functional traits to increase efficiency in resource use. We found that including specific cultivars increased N uptake efficiency and was associated with larger centroid distance in the mixture than in pure culture. As a result, the community level N uptake efficiency in the mixture was also significantly higher than other plant teams, clearly demonstrating the cultivar effects on N resource use. We also showed that in mixtures, N acquisition is more important in increasing productivity in the mixtures than plants'

Table 3

Analysis of variance for uptake efficiency (U_N), grain specific N efficiency ($E_{N.g}$), grain N concentration at final harvest ($C_{N.g}$), grain yield (Y), and nitrogen accumulation efficiency (NAE) for faba bean-wheat and pea-barley plant teams analyzed at community level (summed for each cereal-legume combination). The cultivars were subjected to analysis of variance with community (Plant team), nitrogen level (N-level) and their interaction as factors. The values presented are P. values with the symbols showing significant levels;*** = $P \leq 0.001$; ** = $P \leq 0.01$; * = $P \leq 0.05$; ns = non-significant for all plant teams of each component.

	2017					2018				
	U_N	$E_{N.g}$	$C_{N.g}$	NAE	Y	U_N	$E_{N.g}$	$C_{N.g}$	NAE	Y
Faba bean-wheat										
Fuego-Diskett	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Fuego-Alderon	ns	ns	ns	ns	<.001***	ns	ns	ns	ns	ns
Fuego-Cornetto	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Boxer-Diskett	<.001***	ns	ns	ns	ns	ns	ns	ns	ns	ns
Boxer-Alderon	ns	ns	ns	ns	<.001***	ns	ns	ns	ns	ns
Boxer-Cornetto	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Pea-barley										
Ingrid-Planet	ns	ns	ns	ns	ns	ns	ns	ns	ns	<.001***
Ingrid-Tamtam	ns	ns	ns	ns	ns	ns	ns	ns	ns	<.001***
Ingrid-Vilgott	ns	ns	ns	ns	ns	0.001***	ns	ns	0.007**	<.001***
Clara-Planet	ns	ns	ns	ns	ns	ns	ns	0.013*	ns	ns
Clara-Tamtam	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Clara-Vilgott	ns	ns	ns	ns	ns	ns	ns	ns	ns	<.001***

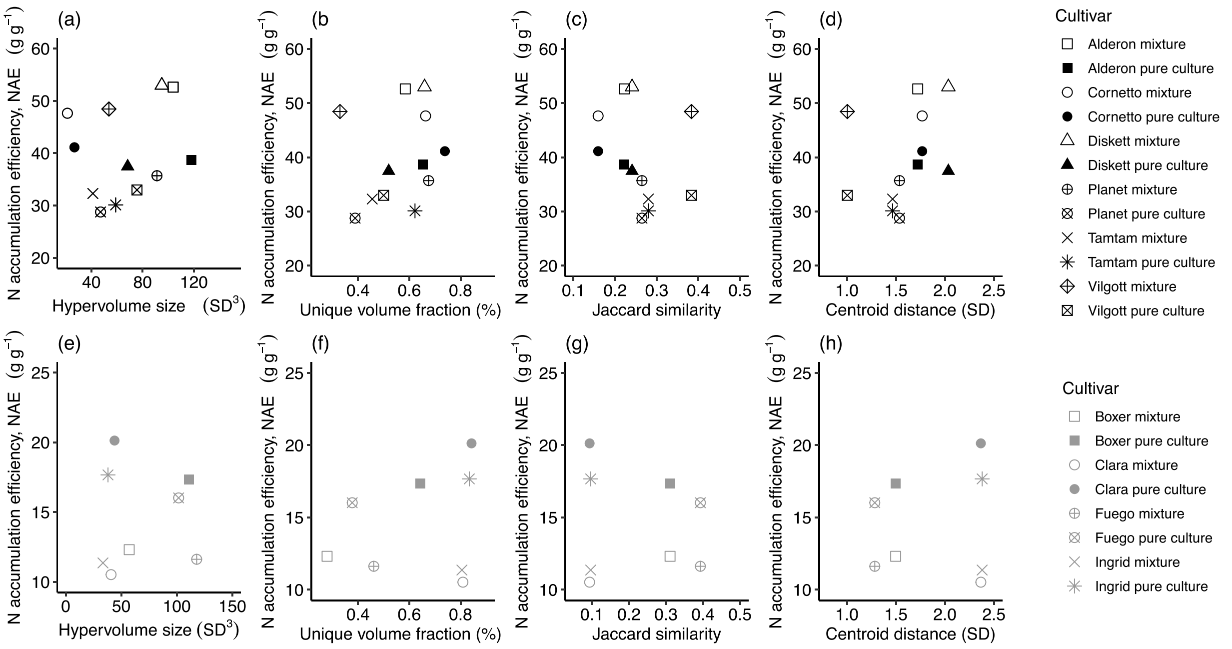

Fig. 5. The relationship between nitrogen accumulation efficiency, NAE and different measures of trait space for cereals (black symbols) and legumes (gray symbols) grown in pure cultures and mixtures. All wheat and barley cultivars in the mixtures were grown with faba bean cv. 'Fuego' and pea cv. 'Ingrid' as legume partners respectively while all faba bean and pea cultivars were grown with wheat cv. 'Diskett' and barley 'Planet' as cereal partners respectively. The points are means (n = 11 for trait space, and n = 16 for NAE) of each crop cultivar at different crop mixing levels and NAE values were averaged across the two years.

ability to convert the acquired N into yield. This clearly shows the mixture effect of the cereal-legumes on N use. The observed N acquisition-conversion pattern is in line with our second hypothesis, *H2*, of greater acquisition and larger trait space in mixtures, and also the findings by Stomph et al. (2019). In most cases, enhanced N uptake is attributed to below ground functional traits such as root length density, and lateral root expansion. Although we lack evidence of the effect of below ground traits on N uptake efficiency, the mean above ground plant N content during the growing season and the seed N enabled us to indirectly quantify the possible contribution of N uptake by the roots. Since functional traits play an important role in the resource use of individuals in interaction (Benavides et al., 2019; McGill et al., 2006), a larger trait space indicates niche complementary, facilitating more efficient resource availability and/or uptake by individuals grown in a species mixture.

The high N uptake resulted in high overall efficiency of N accumulation (NAE). In turn, higher NAE was associated with larger functional traits space, particularly in cereals, which had larger volumes with lower similarity, and with larger distance between the centroids of hypervolumes across the crop mixing levels. In general, a higher diversity (sensu crop mixing) in the cereals facilitated a more efficient N resource use even where minimal trait space differences were realized. This pattern is attributed to the multiple traits used in the evaluation of trait spaces, where changes (for example as a result of mixing) in one trait may have effects on the overall resource capture in the mixture. In crop mixtures, available resources are used more effectively by species with different expressions of functional traits – e.g., less similar and more distant hypervolumes –, because this difference in functional traits (and greater trait space) can indicate niche partitioning (Pigot et al., 2016). Consequently, more species diverse systems acquire more resources due

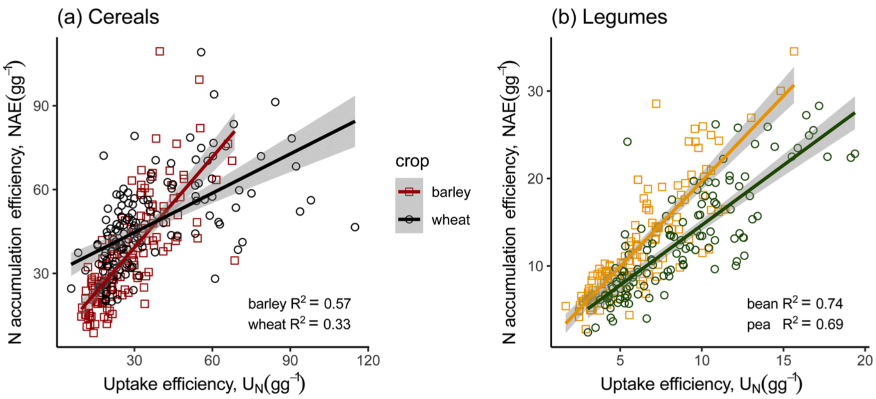

Fig. 6. Regression for nitrogen uptake efficiency (U_N) and nitrogen accumulation efficiency (NAE) for (a) cereals and (b) legumes pooled for both 2017 and 2018 growing seasons. Lines indicate linear regressions: barley: $y = 1.1x + 6.8$, $n = 142$, wheat: $y = 0.47x + 31$, $n = 139$, faba bean: $y = 2.0x + 0.19$, $n = 123$ and pea: $y = 1.4x + 0.96$, $n = 126$. All slopes were significant at $\alpha = 0.05$ with $p \leq 0.0001$.

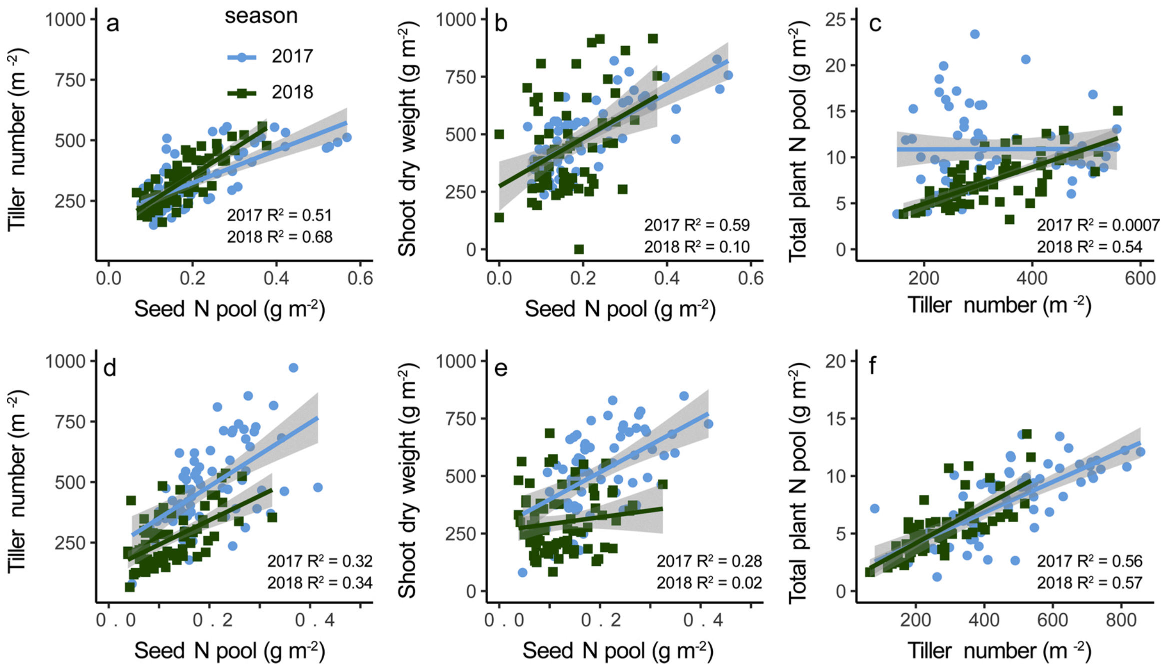

Fig. 7. Regression analysis for seed N pool and tiller number, seed N pool and shoot dry weight, and tiller number and total plant N pool for wheat (a-c) and barley (d-f) at crop flowering in 2017 and 2018. The seed N pools were calculated from seed N contents (g grain^{-1}) of sown seeds. Lines indicate linear regressions: wheat; (a) $y = 710x + 180$; $p < 0.001$ for 2017 and $y=1100x + 140$; $p < 0.001$ for 2018 (b) $y= 1100x + 270$, $p < 0.001$ for 2017 and $y=1100x + 310$, $p < 0.006$ for 2018, (c) $y=0.0011x+11$ for 2017, $p = 0.8$ and $y=0.02x+0.98$, $p \leq 0.001$ for 2018, barley; (d) $y= 1300x + 220$, $p < 0.001$ for 2017 and $y=1000x+ 140$, $p < 0.001$ for 2018 (e) $y=1200x +280$, $p < 0.001$ for 2017 and $y=300x + 260$, $p = 0.257$ for 2018 (f) $y = 0.014x+ 1.3$, $p \leq 0.001$ for 2017 and $y=0.016x+0.93$, $p \leq 0.001$ for 2018, $n = 72$ in all cases.

to complementarity and reduced competition (Li et al., 2013; Stomph et al., 2019). At community level, a combination of traits that make up a phenotype are of greater importance than single traits acting in isolation, making multi-dimensional trait analysis such as hypervolumes a valuable tool in studying specific traits responsible for the superior performance of particular plant teams (Blonder et al., 2014; Bonser, 2006)

4.3. Seed N pool but not soil N enhances above ground functional traits of cereals in mixtures

We found no evidence for strong effects of different soil N levels on N accumulation and use, because the corresponding traits (e.g., NAE and its components) were similar under the treatments with and without additional N. It is likely that N was sufficiently available in the soil prior to the N treatment, given the high level of mineral N in the top 0.3 m soil layer prior to fertilization. High mineral soil N content is known to limit biological N fixation in legumes, which can decrease the potential benefit of growing cereal-legume mixtures (Voisin et al., 2002). Measurements in a pea-barley mixture in the same experiment revealed that additional N did not affect biological N fixation which was comparably low suggesting a limitation of biological N fixation (Jäck et al., 2021). This can partly explain why we found less influence of crop mixing. Therefore, plant growth could have been limited, instead of directly by N availability, by low water supply hampering N uptake. This has been shown to pose substantial effects on N uptake (Nawaz et al., 2012); and one growing season (2018) was indeed dry. In contrast to soil N availability, larger seed N pool resulted in higher accumulated shoot biomass and number of productive tillers in cereals, confirming our third hypothesis, *H3*, that initial seed N pool influences above ground functional

Table 4

Pearson's correlations for tiller number (m^{-2}) and shoot dry weight ($g\ m^{-2}$) against mean N pool during the season (N'), uptake efficiency (U_N), grain specific N efficiency ($E_{N.g}$), grain N concentration at final harvest ($C_{N.g}$) and nitrogen accumulation efficiency (NAE) for wheat and barley grown in 2017 and 2018 seasons. Symbols show results with significant levels;*** = $P \leq 0.001$; ** = $P \leq 0.01$; * = $P \leq 0.05$.

NAE component	Year	Tiller number (m^{-2})				Shoot dry weight (gm^{-2})			
		Wheat		Barley		Wheat		Barley	
		r	P value	*r*	P value	*r*	P value	*r*	P value
N'	2017	0.272	0.024*	0.788	<0.001***	0.436	<0.001***	0.815	<0.001***
	2018	0.796	<0.001***	0.809	<0.001***	0.628	<0.001***	0.596	<0.001***
U_N	2017	−0.458	0.001**	0.262	0.028*	−0.001	0.001**	0.390	0.001**
	2018	−0.118	0.332	0.273	0.020*	0.007	0.953	0.148	0.214
$E_{N.g}$	2017	0.298	0.013*	−0.083	0.494	0.234	0.052	−0.267	0.025*
	2018	−0.050	0.680	−0.100	0.400	0.136	0.260	0.356	0.002**
$C_{N.g}$	2017	0.037	0.758	−0.346	0.003**	−0.084	0.489	−0.238	0.044*
	2018	−0.058	0.639	−0.321	0.006**	0.049	0.685	−0.009	0.943
NAE	2017	0.186	0.136	0.155	0.202	−0.290	0.016*	0.168	0.165
	2018	−0.197	0.108	0.045	0.707	0.241	0.045*	0.322	0.006**

traits through N accumulation, tiller, and shoot biomass production. In our evaluation of N resource use, seed N pool is a strongly influential component of N uptake efficiency, U_N (eq. 1) and partly contributed to the "Nitrogen Pool" trait axis of the hypervolume analysis. Therefore, differences in seed N content between the different cultivars likely influenced N exploitation in the different species components, specifically the cereals in the mixture. In line with this, the cereals consistently had higher N uptake efficiency and NAE in the mixtures than in the pure cultures. In contrast, we found that the contribution of seed N to tiller and shoot biomass production was more in pure culture than in the mixture. It is likely that cultivars with low and high seed N contents, and not the crop mixing, facilitated low and high initial seedling vigour, respectively, which could provide a mechanism for linking N economy and growth in young cereal seedlings. The effects of seedling trait variations among different species are not restricted to seed germination, because the ecological strategies exhibited at seedling stage can also be expressed later in the season when plants are mature (Larson et al., 2020). Although traits at seedling level were not quantified here, we consistently found that hypervolume size, unique volume fraction and Jaccard similarity depended on the cultivar used indicating that seed N contents of the different cultivars may have had an influence. A plausible mechanism could be that, depending on the cultivar, enhanced seed N content stimulated leaf initiation from the apical meristem and other leaf growth processes at seedling stage (Naegle et al., 2005). And for small-seeded plants such as cereals, more efficient resource capture can result from thin young tissues that have greater resource capture per unit of mass (Larson et al., 2020). As a result, higher initial vigour could have been achieved, which in turn is expected to influence later growth and development of key functional traits important in plant-plant interactions. For example, seed N pool is known to boost tiller number at early growth stages (Wang et al., 2014). In crop mixtures, initial vigour may result in higher interspecific competition especially if one species is more vigorous/competitive than the other. This is advantageous for competition against weeds but may also negatively influence the admixed crop species in the mixture (Bertholdsson, 2005; Corre-Hellou et al., 2011). In our case, the cereals were more favoured than the legumes in the mixture when grain yields were compared. In general, we have illustrated that the seed N pool is important in shaping crop species interaction in the mixture, through influencing functional traits responsible for efficient N resource use, not just at the initial but later stages of plant growth.

4.4. *Trade-offs and limitations to resource capture in crop mixtures*

The benefits of greater diversity in the mixtures are currently being exploited with the existing species and/or cultivars on the market, which usually are bred by targeting few traits for better performance (mostly yield) in pure culture. The drawback to the use of these species/cultivars is that no single cultivar possesses all desirable traits since there are trade-offs at individual level (Barot et al., 2017). There is currently much discussion on whether breeding programs specifically targeted towards crop mixtures would allow identifying ideotypes with interaction traits for maximum resource acquisition (Furbank et al., 2019; Litrico and Violle, 2015). Through trait-based approaches, the trade-offs in individual species or cultivars could be addressed by developing species or cultivar mixtures that are more resource efficient and better yielding (Barot et al., 2017). This could be facilitated by a combination of high throughput screening and citizen science initiatives that enhance acquisition of functional trait data that synthesize inter- and intra-specific trait variation (Isaac and Martin, 2019). In addition, suitable crop growth models could be used for the identification of trait combinations and management practices that confer a yield advantage in mixtures (Berghuijs et al., 2020). With these approaches in place, current challenges of maximizing limited resources for growth, and trade-offs between resource acquisition and conversion between species mixtures could be addressed. For example, in our study, the wheat in mixture enhanced N acquisition through high uptake efficiency, U_N, and generally had larger trait spaces with lower overlap but were poor in N conversion (low $E_{N.g}$ values); the opposite occurred in pure cultures. The generally lower N uptake and accumulation coupled to low grain yield in 2018 compared to 2017 can be explained by the warmer temperatures and greater water limitation, in particular earlier in the season, before flowering. Soil water constraint likely limited crop growth in 2018, either directly through reduced transpiration (Tardieu, 2005) or indirectly through reduced photosynthesis, root development and nutrient uptake. N uptake was of particular interest to us because N uptake efficiency of cereals was higher in the mixture than pure cultures despite generally lower values than in 2017, illustrating the interactive effect of N and water availability. Yield reduction, especially in cereals, has been associated with the direct effect of temperature increase (Gammans et al., 2017; Zhao et al., 2017), and indirect effects through increased N immobilization and reduced plant N uptake and use (Hou et al., 2018; Kuzyakov and Xu, 2013; Porter and Gawith, 1999). The temperature increase and reduced plant N uptake was more related to the observed patterns in our study. This makes it relevant in the climate change perspective since the occurrence of weather extremes such as high temperatures and long periods without precipitation experienced in 2018 is predicted to become more frequent in the future in Central and Northern Europe (Toreti et al., 2019).

5. Conclusions

We applied niche-based ecological concepts that relate functional traits to N resource use in mixtures to better understand how crop mixing influences acquisition and conversion of N resources as driven by functional traits. These are relevant for N input management in cereal-legume mixtures, which has wider positive implications in terms of maximizing the systems for productivity. Overall, this study demonstrated that the use of mixture components with high N acquisition and conversion efficiencies, as determined by their functional traits (seed N pool, tiller/branch number, total plant N pool, shoot dry weight, and grain yield), are important in the performance of cereal-legume mixtures. In this study, the cereal components were more efficient in N uptake and compensated for the legume performance in the plant teams at community level depending on the weather conditions, which contrasted between years. Specifically, we found that growing different species at different crop mixing levels had no direct influence on functional trait space, but the choice of cultivar in both pea-barley and wheat-faba bean plant teams had an effect (*H1*), suggesting the need to pay attention in cultivar selection while designing crop mixtures. In addition, we demonstrated that resource acquisition through an increased N uptake in a mixture (*H2*) is associated with a reduced overlap in niche-space in the mixtures; and pure cultures with low resource acquisition capacity, but with better ability to utilize the acquired resource, are still capable of attaining high yields. Finally, we demonstrated that initial seed N pools, which partly contribute to initial seedling growth and vigour, play a significant role in accumulating plant N pools and expression of above ground functional traits (*H3*), which offers a possible mechanistic explanation for the different cultivar performances in the plant teams investigated here.

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Authors' contributions

MW and JA conceived the ideas and designed methodology; JA and OJ collected the data; JA analysed the data; JA led the writing of the manuscript with substantial input from OJ, GV and MW. All authors contributed critically to the drafts and gave final approval for publication.

Data availability statement

The data that support the findings of this study are openly available in figshare, at http://doi.org/10.6084/m9.figshare.14564571

Declaration of Competing Interest

The authors declare that they do not have any conflict of interest

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ppees.2021.125612.

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