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RESEARCH PAPER

Increasing intraspecific diversity of wheat affects plant nutrient contents but not N recovery in the plant-soil system

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ABSTRACT

Crop homogenization in conventional agriculture has been pervasive while ecology has shown positive effects of biodiversity on ecosystem functioning, that arise from complementarity/facilitation and sampling/selection effects. These effects are well documented for interspecific diversity in both natural ecosystems and agro-ecosystems but remain less documented at an intraspecific level, particularly for the rates of nutrient uptake by plants and nutrient losses from ecosystems. We conducted a field experiment with 88 experimental plots cultivated with 1, 2, 4 or 8 wheat varieties and 1, 2, 3 or 4 functional groups to assess the effects of the number of varietal and functional diversity of winter wheat on plant biomass production, plant nutrient contents (N, Ca, Cu, Fe, Mg, Mn, P, K, Na and Zn) and fertilizer N recovery in the plant-soil system using a ^{15}N labeling method. We found both negative and positive effects of the number of varieties or number of functional groups on shoot Cu, Fe, Zn, Na and P contents, but no significant effects of intraspecific diversity on biomass production, N content and ^{15}N recovery in the plant-soil system. Our results show differential responses to an increase of intraspecific diversity of wheat on the contents of several essential nutrients in plants and highlight the need to jointly analyze multiple nutrients. Our study also suggests that increasing intraspecific diversity had no overall negative effects on biomass production or N content. Using knowledge on variety functional traits to target specific complementarity mechanisms when designing variety mixtures could thus lead to a positive effect on nutrient absorption and biomass production.

Introduction

Intensive cropping systems are based on monocultures grown in pure stands, leading to genetic and spatial homogenization of agroecosystems (Bonnin et al., 2014; Khoury et al., 2022). These agricultural practices are coupled with a broad use of synthetic inputs, including mineral fertilizers, which induce the rise of nitrous oxide (N_2O) emissions, a potent greenhouse gas, water pollution and eutrophication of aquatic ecosystems, with damaging consequences on biodiversity (Barnard et al., 2005; Brauman et al., 2020; Campbell et al., 2017; Carpenter et al., 1998; van Groenigen et al., 2015). Furthermore, it is now well established that intensive cropping systems, despite their high yields, lack resilience because of their dependency on fossil fuels and their vulnerability against pests, diseases, and extreme climatic events (Østergård et al., 2009). As pleaded by numerous authors, it is therefore crucial to find more sustainable agricultural practices, and increasing plant biodiversity within agroecosystems would be an important avenue to reach this goal (Gaba et al., 2015; Isbell et al., 2017; Tilman et al., 2001; Vialatte et al., 2021).

Emerging from empirical studies and mathematical models, the Biodiversity and Ecosystem Functioning (BEF) paradigm predicts a positive relationship between increasing plant diversity and ecosystem ability to produce biomass, capture resources, decompose and recycle essential nutrients (Cardinale et al., 2012; Tilman et al., 2014). Fostering plant interspecific diversity improves biomass production (Hector et al., 1999; Jiang et al., 2021; Tilman et al., 1997), but also leads to better

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nitrogen (N) and phosphorus (P) exploitation by plants as well as a better retention of these nutrients in soil (Han et al., 2021; Oelmann et al., 2011a, 2011b; Tilman et al., 1996, 1997; Hooper & Vitousek, 1997; Lama et al., 2020). These beneficial effects of biodiversity are generally attributed to two distinct mechanisms: the so-called *selection effect* and the *complementarity effect* (Hughes et al., 2008; Loreau & Hector, 2001). The *selection effect* is based on the *sampling effect*, i.e. the fact that a diverse community is statistically more likely to include species that perform particularly well (e.g., in terms of biomass) because they are well adapted to the environmental conditions. For this mechanism to hold, these fittest species must be able to dominate the community (e.g., in terms of number of individuals or surface occupied) and thus to drive upward the performance of the community. The *complementarity effect* requires positive interactions between species (*facilitation effect*) or niche partitioning between species due to functional complementarity that leads to a more efficient exploitation of resources by the community (Cardinale et al., 2012). These mechanisms emerge from disparities among species characteristics within a community, that generate functional diversity (Roscher et al., 2012; Tilman et al., 1997). Such diversity can be assessed using the variability of functional traits within functional groups, but species richness can independently influence biomass accumulation due to substantial differences in species characteristics within functional group (Reich et al., 2004).

The BEF framework can be applied to agroecosystem management in order to promote the delivery of multiple ecosystem services (Beillouin et al., 2021; Finney & Kaye, 2017). In particular, interspecific diversity in agroecosystems can promote over-yielding (i.e. diverse communities can be more productive than the mean production of monocultures) and long-term soil fertility through an increase in the absorption of mineral N and a decrease in N losses (Beillouin et al., 2021; Isbell et al., 2017). Furthermore, other nutrients essential to plant growth may be influenced by plant diversity in agroecosystems, as observed for P and potassium (K) acquisition that increased when wheat was mixed with barley (Zhang et al., 2017).

In comparison to crop species, crop varieties may have a lower traits variability, but traits can still differ substantially between varieties, especially for no- or low-selected functional traits (Cantarel et al., 2021; Hughes et al., 2008; Litrico & Violle, 2015). For example, wheat genotypes differ in traits related to N use efficiency and crop performance at different fertilization rates (Barraclough et al., 2014; Ivić et al., 2021; Le Gouis et al., 2000), but also have considerable variations in nutrient concentration and grain protein content (Pandey et al., 2016) and in root traits that are important for nutrient uptake and drought tolerance (Cantarel et al., 2021; Colombo et al., 2022). Therefore, an increase in crop intraspecific diversity may also induce positive effects on yield, yield stability, nutritional quality, stress tolerance, soil fertility and the reduction of soil erosion (Barot et al., 2017; Snyder et al., 2020). Indeed, over-yielding and a better yield stability have already been reported in agroecosystems based on varietal mixtures (Beillouin et al., 2021; Borg et al., 2018; Reiss & Drinkwater, 2018) and some studies also reported positive effects of intraspecific diversity on N and P cycles (Cook-Patton et al., 2011; Jiang et al., 2021). Nevertheless, general knowledge about the effect of variety mixtures is mostly limited to biomass production and to crop resistance to pests and diseases (Beillouin et al., 2021).

Several mechanisms linked to the fate of nutrients in the plant-soil system are expected to increase yield while reducing nutrient loss in varietal mixtures (Barot et al., 2017). Variability in root architecture and nutrient foraging strategy could promote complementarity effects (Lynch & Brown, 2012). Furthermore, host variability leading to niche differentiation for mycorrhizal colonization could promote increase in mycorrhizal diversity and abundance (Taschen et al., 2023), while a facilitation effect can emerge from nutrient transfers through mycorrhizal networks between plant individuals (Wipf et al., 2019). Finally, phenological differences between varieties (Cantarel et al., 2021) may lead to complementarity in the timing of nutrient absorption (Weih et al., 2016) and thus to an extension of the uptake period by mixtures. However, the effect of intraspecific diversity on the ability of crops to exploit nutrients and on the plant-soil system to retain N remains largely unknown and has yet to be tested in field experiments.

To tackle these issues, we studied the impact of intraspecific diversity of winter wheat (*Triticum aestivum*) by manipulating the number of wheat varieties (1, 2, 4, 8) and the number of functional groups of varieties (1, 2, 3, 4) in a field experiment. Our objectives were to assess the effect of both varietal and functional diversity on (i) wheat shoot and root biomass, (ii) the distribution of N in the plant-soil system and the recovery of fertilizer N in the plant-soil system using a ^{15}N tracer, and (iii) shoot nutrient content (Ca, Cu, Fe, Mg, Mn, P, K, Na and Zn).

Materials and methods

Experimental design

A field experiment was conducted in 2014–2015 at the INRAE experimental station in Versailles, France (48°48′26″N, 2°05′13″E) in which the number of varieties (1, 2, 4, 8) and the number of functional groups (1, 2, 3, 4) of winter wheat (*Triticum aestivum*) were manipulated.

Selection of varieties and delineation of functional groups (clusters)

In total, 57 varieties of winter wheat were chosen by screening for a large diversity of functional traits and breeding histories (Cantarel et al., 2021). These varieties included modern elite varieties used in conventional agriculture in the Paris-Basin, lines extracted from multi-parental highly recombinant populations (MAGIC, see Thépot et al. (2015)), modern varieties inbred for organic agriculture, as well as landraces highly cultivated in France in the early 20th century. The varieties went through a phenotyping of 27 above- and below-ground functional traits including morphological, phenological and physiological traits, some of them being correlated with each other (for more details, see Cantarel et al. (2021) and Dubs et al. (2018a)). Four functional groups (hereafter "clusters") were defined by hierarchical classification, based on these functional traits. They mainly differed from each other in terms of disease sensitivity, growth rate, soil exploration and soil exploitation ability. Despite the large panel of functional traits used to construct these four clusters, we cannot exclude that adding other functional traits in our clustering analysis might have led to other functional groups with clusters exploring other aspects of functional diversity. Four varieties were retained within each of the four clusters so that a total of 16 varieties were used for the field experiment (see Appendix A: Tables 1 and 2). The four varieties per cluster were chosen to be representative of their cluster, and we ensured that the four clusters remained robust after sub-sampling of the 16 varieties by using hierarchical clustering and K-means clustering (Dubs et al., 2018a). Cluster 1 (C1) included varieties sensitive to fungal disease, with a high relative growth rate and a low flag leaf nitrogen content; Cluster 2 (C2) included varieties with a low specific root length and low root NO_3^- uptake; Cluster 3 (C3) included tall varieties with high NH_4^+ uptake; Cluster 4 (C4) included varieties with high specific root length and high NO_3^- uptake (see Appendix A: Fig. 1).

Intraspecific diversity treatments

The 16 varieties were associated by crossing different levels of varietal diversity with different levels of functional diversity and were distributed in 88 experimental plots that were 10.5 m wide and 8 m long (see Appendix A: Fig. 2). Varietal diversity varied from monocultures (16 plots) to mixtures of 2 varieties (24 plots), 4 varieties (28 plots) and 8 varieties (30 plots). Functional diversity was represented by the number of clusters present in the plot and varies from 1 cluster (28 plots) to 2 clusters (30 plots), 3 clusters (16 plots) and 4 clusters (14 plots). The treatments were randomly distributed within the experimental field, and the plots were isolated from the others by strips of Triticale (*x*

Triticosecale) 1.75 m wide. Seeds of the different varieties in the mixtures were in equal proportions, and were blended before sowing which means that their distribution along the sowing unit was random. Our objective was to assess the effects of the number of varieties and the number of functional groups, and not to test for differences between mixtures. Hence, the 88 mixtures or monocultures were not replicated, while we used replicates of mixtures having the same number of varieties and/or functional groups. Moreover, the mixture composition was random which means that mixtures were not implemented intentionally to optimize their performance.

Management and timeline of the experiment

The management of the experimental field corresponded to conventional practices with reduced inputs. The target yield was lowered to 2/3 of the maximum estimated yield (i.e. 60 quintal ha^{-1} instead of 90 quintal ha^{-1}) for input calculations. The field was ploughed to 30 cm depth before sowing in November 2014 at a density of 180 seeds m^{-2}. Each plot was composed of six sowing units (1.75 m wide, 8 m long), themselves containing eight sowing lines (8 m long) and equally spaced 17.5 cm apart (see Appendix A: Fig. 3). Seeds were coated with insecticides (CELEST: 0.2 l quintal^{-1} - 25 g l^{-1} of Fluioxonil), fungicides (SIGNAM: 60 g quintal^{-1} - 300 g l^{-1} of Cypermethryne), and herbicides (Archipel® and Harmony Extra®) that were sprayed on 14 March 2015. Then, 140 kg N ha^{-1} of a N fertilizer (ammonium-nitrate NH_4NO_3) was spread in three times from March to May 2015 (40 kg N ha^{-1} on March 5, 60 kg N ha^{-1} on April 16, and 40 kg N ha^{-1} on May 11). All plots were entirely harvested from the last week of July to the first week of August 2015 and the average yield of the experimental field reached 5.95 t ha^{-1} across all plots (Vidal et al., 2020).

Soil characteristics

Soil cores (8 cm diameter x 15 cm depth) were collected in each of the 88 experimental plots, and soil samples were analysed with standardised methods at the Laboratoire d'Analyses des Sols (INRAE, Arras, France) for total N content, total C content, organic matter (OM) content and clay (< 2 μm), silt (2–50 μm) and sand (50–2000 μm) contents. Data were expressed in g kg^{-1} (see Appendix A: Table 3).

Aboveground and belowground plant biomass

Aboveground and belowground plant biomass was collected from 1 to 6 June 2015, at the onset of flowering, inside a sampling area of 52.5 cm x 50 cm, containing 3 sowing lines (see Appendix A: Fig. 3), by uprooting whole plants and by separating shoots from roots. This sampling area was located inside an area of 90 cm by 90 cm where ^{15}N was added (see below). Two soil cores (8 cm diameter x 15 cm depth) were collected within the same sampling area of 52.5 cm x 50 cm, one within the sowing line and one in between, and fine roots were extracted from these soil cores. Roots were washed, and shoot and root materials were dried at 65 °C for 72 h and weighed. Data were expressed in g m^{-2}. Total root biomass was extrapolated to the sampling area based on the roots collected within and between the sowing lines. Total plant biomass was estimated as the sum of shoot and root biomass, and the root:shoot biomass ratio was calculated.

^{15}N labeling and measurement of the distribution/recovery of N in the plant-soil system

A ^{15}N tracer was applied on 11 March 2015 on each of the 88 experimental plots, by adding 36 mg ^{15}N m^{-2} (i.e. 0.36 kg ^{15}N ha^{-1}) in the form of $^{15}NH_4^{15}NO_3$ (at 98 %) dissolved in 1 L of demineralized water which was sprinkled slowly over an area of 90 cm x 90 cm (see Appendix A: Fig. 3). The ^{15}N addition occurred few days after the first fertilizer addition, in the same form as the fertilizer input and was equivalent to 0.9 % of the fertilizer added (40 kg N ha^{-1} on March 5). Our objective was hence to evaluate the fate of ammonium-nitrate added to the soil.

Soil samples, and shoot and root biomasses were collected inside a sampling area of 52.5 cm x 50 cm within the labeled area of 90 cm x 90 cm to avoid border effects (see above). Soil samples were oven dried 24 h at 105 °C and plant materials were oven dried 72 h at 65 °C. Samples were grounded and analysed by a mass spectrometer (EA-IRMS, Carlo-Erba NA-1500 NC Elemental Analyzer in line with a Fisons Optima Isotope Ratio Mass Spectrometer) for %N and $\delta^{15}N$. Shoot or root N contents were calculated as the product of the N concentration times the dry biomass of the sample. Soil N content was calculated as the product of the N concentration of the soil sample times the estimated mass of soil over 1 m^2 and 15 cm depth. Shoot, root, and soil N contents were expressed in g N m^{-2}. Total plant N content was determined as the sum of shoot and root N contents. In each compartment of the plant-soil system (soil, shoot, root), ^{15}N content (mg ^{15}N m^{-2}) was calculated as the atom% excess ^{15}N concentration (measured atom%^{15}N minus natural abundance of ^{15}N) times the mass of N (g N m^{-2}). The ^{15}N recovery in soil, shoot, and root was determined as the ratio between the ^{15}N content of each compartment and the amount of ^{15}N added, and expressed in %. The total plant ^{15}N recovery was determined as the sum of shoot and root ^{15}N recoveries, and is a proxy of the uptake of the added N by plants.

Shoot nutrient content

In addition to N content, the concentrations of nine essential nutrients - calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), phosphorus (P), potassium (K), sodium (Na) and zinc (Zn) - were measured in the shoot material by radial ICP-AES (Inductive Coupled Plasma coupled to Atom Emission Spectrometry) after dry mineralization at the Unité de Services et de Recherche en Analyses Végétales et Environnementales (INRAE, Bordeaux, France). The contents of these nine nutrients in shoots were calculated as the product of shoot nutrient concentration times shoot dry biomass, and were expressed in g nutrients m^{-2}.

Statistical analysis

All statistical analyses were performed with the R software (R-4.1.1, 2022). The significance level was set to 0.05. Statistical descriptors (min, max, mean, standard deviation) of the response variables are provided in Appendix (see Appendix A: Table 4). As detailed below, we conducted statistical analyses in separate models to assess the effects of the number of varieties, the effects of the number of functional groups, the effects of the means and the variances of six functional traits in the mixtures, and the effect of the presence/absence of the different functional groups in the mixtures on the studied variables.

Analysis of the effect of the number of varieties

The effect of varietal diversity was tested using analysis of variance (ANOVA, package Car - 3.0–11) (4 levels; number of varieties: 1, 2, 4, 8). Prior to analyses, and as some soil characteristics showed a non-random distribution along the experimental field with spatial gradients, a model integrating coordinates and their square values (X, X^2, Y, Y^2) of each experimental plot was conducted on each of the response variables to test for potential spatial gradients. It included a stepwise selection based on AIC criteria (package MASS 7.3–54) to retain the most relevant model. When significant, an analysis was conducted on the residuals of this model to correct for a geographical bias (see Appendix B: Fig. 1). In total, 15 among the 21 response variables showed a significant geographical signal (total plant biomass, shoot biomass, plant N content, shoot N content, root N content, soil N content, root ^{15}N recovery, and shoot Ca, Cu, Fe, Mg, Mn, P, K and Zn contents). In addition, an analysis of covariance (ANCOVA) including the number of varieties and main soil characteristics (the soil clay, silt and organic matter contents) was also conducted (this approach gave qualitatively similar results as the first approach, for more details, see Appendix B: Table 1). Residuals of the ANOVA and ANCOVA were checked for normality (Shapiro test), homoscedasticity (Bartlett test) and independence between the residuals of the models and the predicted values (graphically). Box-Cox transformations (package Car - 3.0–11) were applied when the conditions of ANOVA and ANCOVA were not met and nonparametric Kruskal-Wallis tests were conducted if necessary. When the ANOVA was significant, pairwise comparisons were carried out by Tukey HSD (package emmeans - 1.7.2) or its nonparametric equivalent, the Wilcoxon-Mann-Whitney test (package pgirmess - 1.7.1).

Analysis of the effect of the number of functional groups

The effect of functional diversity was tested following the exact same procedure as for the number of varieties (see above), using an analysis of variance (4 levels; number of functional groups: 1, 2, 3, 4), and an analysis of covariance including the soil parameters in the model (this approach gave qualitatively similar results as the first approach, for more details, see Appendix B: Table 1). In addition, multiple linear regression models including the means and the variances of six functional traits (Flag leaf N content, Specific Root Length, NO_3^- uptake, NH_4^+ uptake, Relative Growth Rate, and Height of the main shoot; see Appendix C: Table 1 and Table 2) in the mixtures were conducted. This second approach using continuous variables to assess the effects of functional diversity is complementary to the analysis of variance performed using the number of functional groups in the mixtures and provides information at the scale of the functional traits.

Analysis of the effect of the presence/absence of the four functional groups in the mixtures

The effect of the presence of the four functional groups (or clusters) in the mixtures was tested using a 4-factor analysis of variance (Cluster 1, 2, 3, 4 with two levels for each cluster: presence/absence). Then, pairwise comparisons between the two levels of each functional group (with or without) were performed by *t*-tests (package rstatix - 0.7.0) for the variables for which the analysis of variance indicated significant effects. P-values were adjusted by the Bonferroni-Holm method (package stats - 4.1.1).

Results

Effect of intraspecific diversity on wheat biomass

Wheat biomass ranged from 1007 g m^{-2} to 1954 g m^{-2}, and averaged 1351 ± 201 g m^{-2} (mean ± standard deviation) across all plots. Shoot biomass averaged 1248 ± 186 g m^{-2} and root biomass 103 ± 29 g m^{-2} (see Appendix A: Table 4). The number of varieties (varietal diversity), the number of functional groups (functional diversity) and the means and variances of the six functional traits investigated had no significant effect on shoot biomass, root biomass, and total plant biomass, nor on the root:shoot ratio (Table 1, Fig. 1, see Appendix B: Table 1 and Appendix C: Table 1 and Table 2).

Effect of intraspecific diversity on N distribution and ^{15}N recovery in the plant-soil system

Plant N content ranged from 7.48 g N m^{-2} to 17.51 g N m^{-2}, with an average of 11.5 ± 1.9 g N m^{-2}. The percent recovery of ^{15}N in plant biomass ranged from 22.4 % to 46.4 %, with an average of 31.9 ± 5.2 % (see Appendix A: Table 4). No significant effect of the number of varieties, the number of functional groups and the means and variances of the six functional traits investigated was observed on shoot, root, and total plant N contents nor on shoot, root and total plant ^{15}N recovery (Table 1, Fig. 2A, see Appendix B: Table 1 and Appendix C: Table 1 and Table 2).

Soil N content varied from 141 g N m^{-2} to 264 g N m^{-2}, with an average of 174.8 ± 24.5 g N m^{-2}. The percent recovery of ^{15}N in soil ranged from 10.4 % to 36.0 % of ^{15}N, with an average of 18.9 ± 4.7 % (see Appendix A: Table 4). No significant effect of the number of varieties, the number of functional groups, and the means and variances of the six functional traits investigated was observed on soil N content and soil ^{15}N recovery (Table 1, Fig. 2B, see Appendix B: Table 1, and Appendix C: Table 1 and Table 2).

Table 1

Results of the analysis of variance testing for an effect of the number of varieties and of the number of functional groups (or clusters) on the studied variables.

		Varietal diversity (number of varieties)			Functional diversity (number of clusters)		
	Variable	Fisher or Chi-square ^{KW}	P-value	adj. R^2	Fisher or Chi-square ^{KW}	P-value	adj. R^2
Biomass production	Plant biomass ^{R}	0.44	0.72	–	0.41	0.75	
	shoot ^{R}	0.68	0.56	–	0.34	0.79	–
	root	0.55 ^{BC}	0.65	–	1.28 ^{BC}	0.29	–
	Root: shoot ratio	3.42 ^{KW}	0.33	/	0.82 ^{BC}	0.49	–
N distribution in the plant-soil system	Plant N content ^{R}	0.61	0.61	–	0.90	0.45	–
	shoot ^{R}	0.88	0.46	–	0.77	0.51	–
	root ^{R}	0.87 ^{BC}	0.46	–	0.8 ^{BC}	0.50	–
	Soil N content ^{R}	2.42 ^{KW}	0.49	/	0.37 ^{KW}	0.95	/
^{15}N recovery in the plant-soil system	Plant ^{15}N recovery	1.67 ^{BC}	0.18	–	0.97 ^{BC}	0.41	–
	shoot	1.98 ^{BC}	0.12	–	0.91 ^{BC}	0.44	–
	root ^{R}	1.1 ^{BC}	0.35	–	0.96 ^{BC}	0.41	–
	Soil ^{15}N recovery	1.52 ^{KW}	0.68	/	0.05 ^{BC}	0.99	–
Shoot nutrient content	Ca ^{R}	2.13	0.10	–	1.24	0.30	–
	Cu ^{R}	2.80	0.05	0.06	0.99	0.40	–
	Fe ^{R}	6.77 ^{BC}	0.0004	0.17	2.05 ^{BC}	0.11	–
	Mg ^{R}	5.44 ^{KW}	0.14	/	3.95 ^{KW}	0.27	/
	Mn ^{R}	7.64 ^{KW}	0.05	/	1.38 ^{BC}	0.25	–
	P ^{R}	1.91	0.13	–	2.99	0.04	0.06
	K ^{R}	0.76	0.52	–	1.03	0.38	–
	Na	15.06 ^{KW}	0.002	/	0.22	0.89	–
	Zn ^{R}	3.13 ^{BC}	0.03	0.07	2.26	0.09	/

^{R} indicates variables for which analyses were made on the residuals of a spatial model (geographical correction). ^{BC} indicates Box Cox transformation. ^{KW} indicates when ANOVA was substituted to non-parametric Kruskal-Wallis test, when ANOVA application conditions were not met. P-values are given for 3 degrees of freedom.

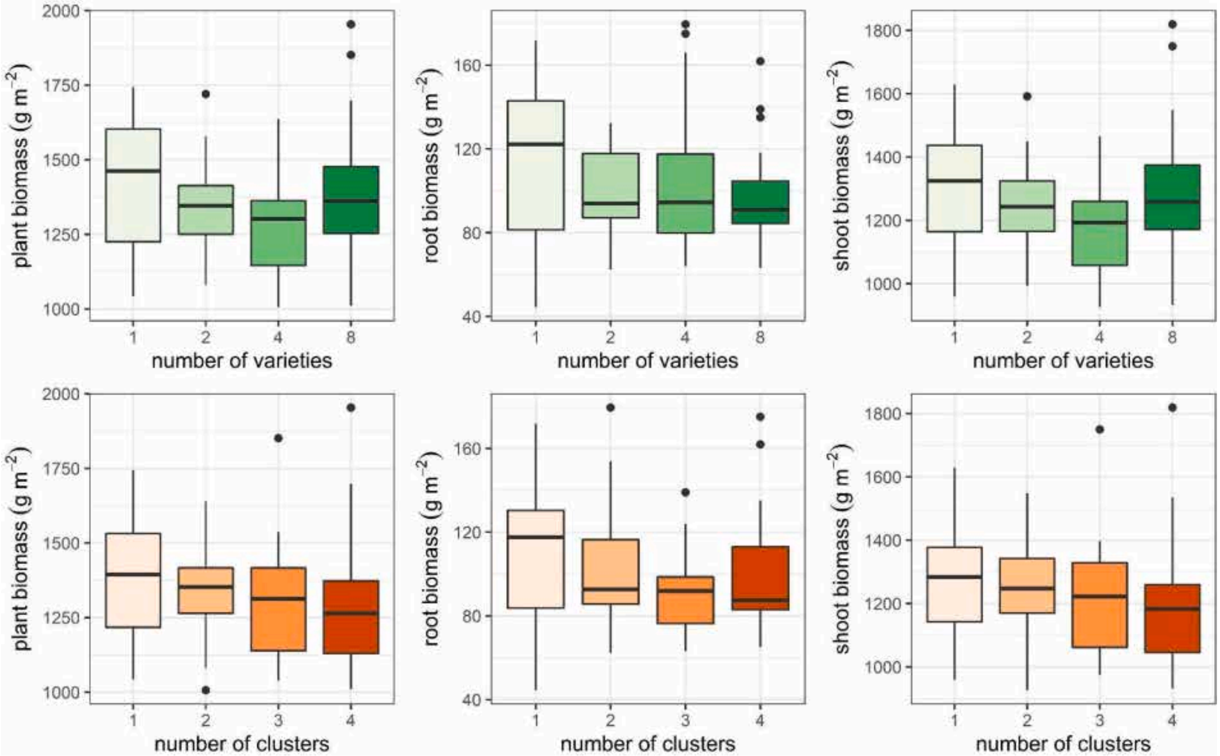

Fig. 1. Effect of the number of varieties (upper part, in green) and the number of functional groups (or clusters, bottom part, in orange) on total plant biomass, root biomass and shoot biomass of winter wheat.

Effect of intraspecific diversity on shoot nutrient content

Statistical descriptors of shoot nutrient content are shown in Appendix A: Table 4. The number of varieties had a significant effect on shoot Cu content ($p = 0.05$, adj. $R^2 = 0.06$), shoot Fe content ($p = 0.0004$, adj. $R^2 = 0.17$; similarly the analysis of covariance including the soil characteristics revealed a significant effect of the number of varieties on shoot Fe content, see Appendix B: Table 1), shoot Zn content ($p = 0.002$, adj. $R^2 = 0.07$), and shoot Na content ($p = 0.03$, adj. $R^2 = 0.06$) (Table 1, Fig. 3A). Specifically, monocultures had a significantly higher shoot Fe content than mixtures with 8 varieties, and mixtures with 2 varieties had a higher shoot Fe content than mixtures with 4 or 8 varieties. In addition, mixtures with 2 varieties had a significantly higher shoot Zn content than mixtures with 8 varieties (Fig. 3A). Besides, mixtures with 2 varieties had a significantly lower shoot Na content than mixtures with 8 varieties (Fig. 3A). Post-hoc tests revealed no significant differences between the number of varieties for shoot Cu content. The number of varieties had no significant effect on shoot Ca, Mg, Mn, P and K contents.

The number of functional groups had a significant effect on shoot P content ($p = 0.04$, adj. $R^2 = 0.06$; similarly the analysis of covariance including the soil characteristics revealed a significant effect of the number of functional groups on shoot P content, see Appendix B: Table 1) (Table 1, Fig. 3B), but post-hoc tests did not reveal any significant difference between the numbers of functional groups on shoot P content. The number of functional groups had no significant effect on shoot Ca, Cu, Fe, Mg, Mn, K, Na and Zn contents (Table 1).

The variance of plant NH_4^+ uptake had a significant negative effect on shoot Cu content ($p = 0.02$, adj. $R^2 = 0.10$), Fe content ($p = 0.004$, adj. $R^2 = 0.14$), Mg content ($p = 0.02$, adj. $R^2 = 0.10$) and Zn content ($p = 0.02$, adj. $R^2 = 0.09$) (see Appendix C: Table 1). The mean of the height of the main shoot had a significant positive effect on shoot K content ($p = 0.0002$, adj. $R^2 = 0.22$) (see Appendix C: Table 2). The means and variances of the other traits investigated had no significant effect on the shoot nutrient contents.

Effect of the identity of the functional group present in the mixture

A significant effect of the identity of the functional groups (or clusters) present in the mixtures was detected on root biomass ($p = 0.01$, adj. $R^2 = 0.10$), shoot Mg content ($p = 0.01$, adj. $R^2 = 0.10$), shoot P content ($p = 0.03$, adj. $R^2 = 0.07$) and shoot K content ($p = 0.0003$, adj. $R^2 = 0.19$) (see Appendix C: Table 3).

The presence of Cluster 1 significantly decreased root biomass by 19.9 ± 11.9 g m^{-2} (95 % Confidence Interval, or CI, $p = 0.005$), and shoot K content by 1.9 ± 1.1 g K m^{-2} (95 % CI, $p = 0.002$), while the presence of Cluster 3 significantly decreased shoot Mg content by 0.09 ± 0.06 g Mg m^{-2} (95 % CI, $p = 0.01$) (Fig. 4; see Appendix C: Table 3).

Discussion

Contrary to our assumptions, varietal and functional diversities had no significant effect on wheat biomass, N content, ^{15}N recovery and on the shoot nutrient content for four of the nine other nutrients measured. Furthermore, the number of varieties or of functional groups of varieties had no effect on soil N content and soil ^{15}N recovery. In contrast, the number of varieties significantly affected shoot Cu, Fe, Na and Zn contents, while the number of functional groups significantly altered shoot P content. Overall, these effects of varietal or functional diversity explained a small part of the observed variance, as indicated by low R^2 values (Table 1). In our experimental design, the number of varieties and the number of functional groups are partly nested (e.g. mixtures with a high number of functional groups necessarily also contained a high number of varieties, while the reverse is not necessarily true), hence the effects of the two factors are not independent, but overall consistent.

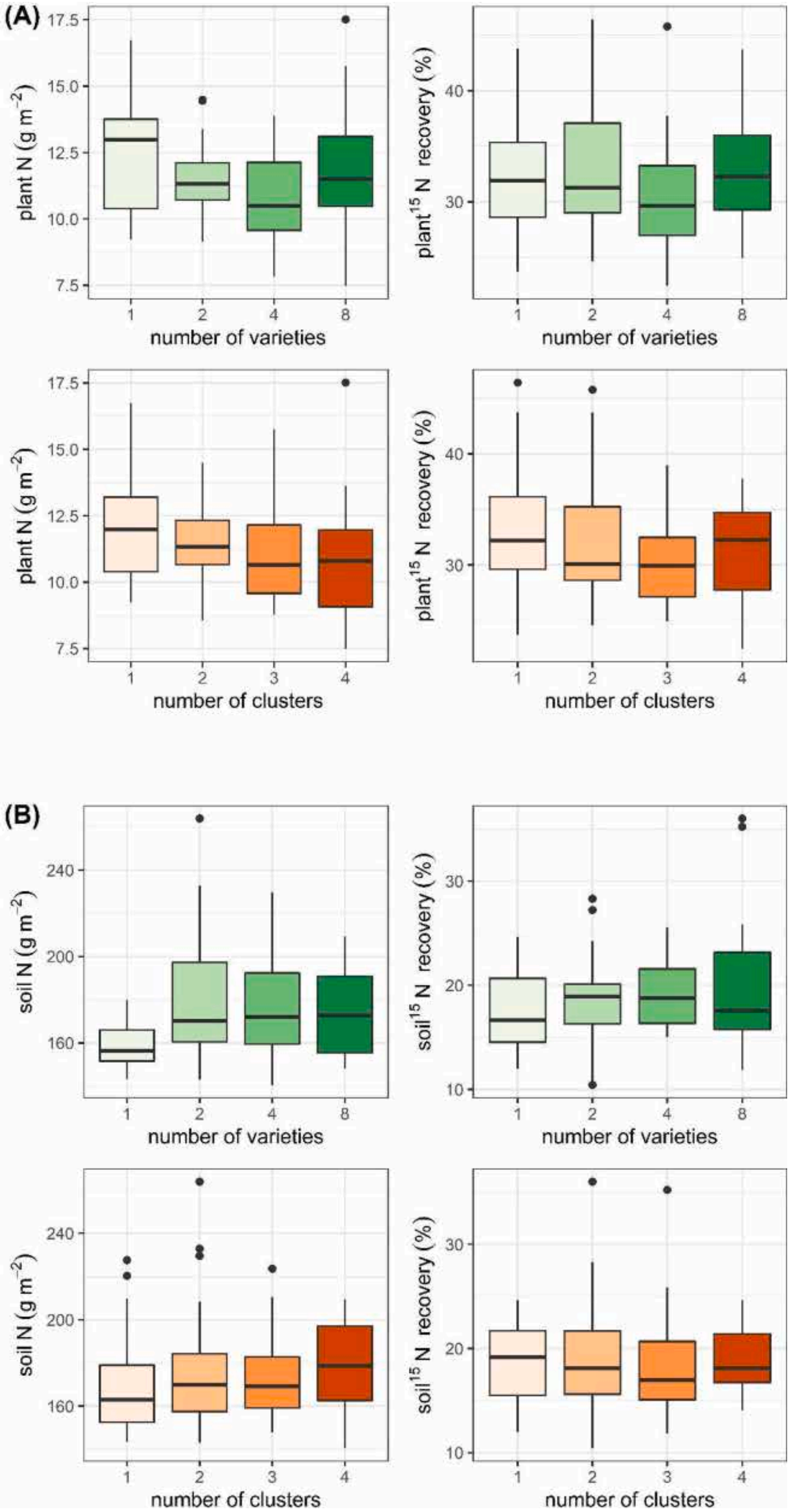

Fig. 2. Effect of the number of varieties (upper part, in green) and the number of functional groups (or clusters, bottom part, in orange) on plant N content and plant ^{15}N recovery (panel A), and on soil N content and soil ^{15}N recovery (panel B).

Interpretation of the absence or weakness of intraspecific diversity effects

Overall, we found no evidence for effects of intraspecific diversity on most of the variables measured. In particular, our results suggest no effect of intraspecific diversity on the plants' efficiency to exploit N added by fertilization, in agreement with the lack of response of wheat biomass production, and no effect of intraspecific diversity on N recovery in the soil. Consistently, most studies conducted on the same

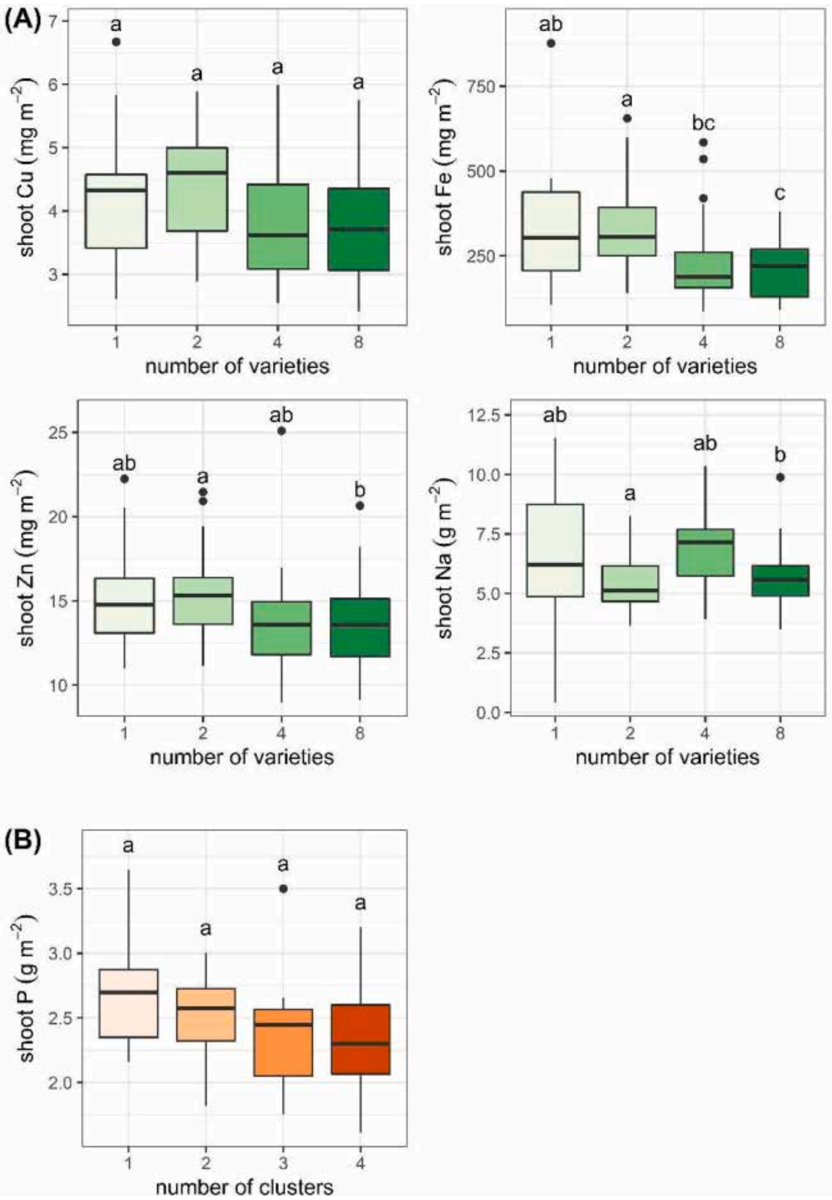

Fig. 3. Effect of the number of varieties on shoot Cu, Fe, Zn and Na contents (panel A), and of the number of functional groups (or clusters) on shoot P content (panel B). Different letters indicate significant differences between the different levels of intraspecific diversity (post-hoc tests).

experimental design have reported no or little effect of the number of varieties and of the number of functional groups on other response variables, including epigeal and hypogeal biodiversity (predatory arthropods (Dubs et al., 2018b), earthworms (Chassé et al., 2019), and collembola (Salmon et al., 2021)), and on yield and susceptibility to fungal diseases of wheat (Vidal et al., 2020). However, a study conducted in 2016 also assessing the effects of wheat variety diversity in an adjacent area of the present study reported a positive effect of the number of varieties on the abundance of Arbuscular Mycorrhizal Fungi (AMF) and on the activity of leucine amino-peptidase (LAP, an enzyme involved in organic N mineralisation), while no effects of intraspecific diversity were detected on phosphatase activity (Taschen et al., 2023). More generally, two meta-analyses on wheat and other crops showed a non-transgressive but positive over-yielding induced by varietal diversity (Borg et al., 2018; Reiss & Drinkwater, 2018).

The literature on diversity effects on nutrient pools is scarce and largely limited to interspecific diversity, especially for other nutrients than N. A positive effect of interspecific diversity has been reported on plants amount of P, K, Ca and Mg in a constructed wetland (Han et al., 2021), P exploitation by grassland plants (Oelmann et al., 2011b), and amount of available P and phosphatase activity in soils of a semi-arid temperate steppe (Jiang et al., 2021). In contrast, other studies reported no significant effect of interspecific functional diversity on ^{15}N recovery by plants or by the soil in a Mediterranean grassland (Hooper & Vitousek, 1997), and a decrease of shoot P concentration with interspecific diversity relative to N and C concentrations in a temperate grassland (Abbas et al., 2013).

As for many studies in ecology, the statistical power associated with our experimental design might have been too small to detect significant effects of intraspecific diversity. This issue has already been encountered at the interspecific scale (Lama et al., 2020), and mentioned as a possible explanation for no or little effect of intraspecific diversity on variables measured in the same field experiment (Dubs et al., 2018b; Salmon et al., 2021). However, other mechanisms might be in play, such as insufficient levels of diversity, a duration of the experiment of only a year and/or insufficiently stressful conditions.

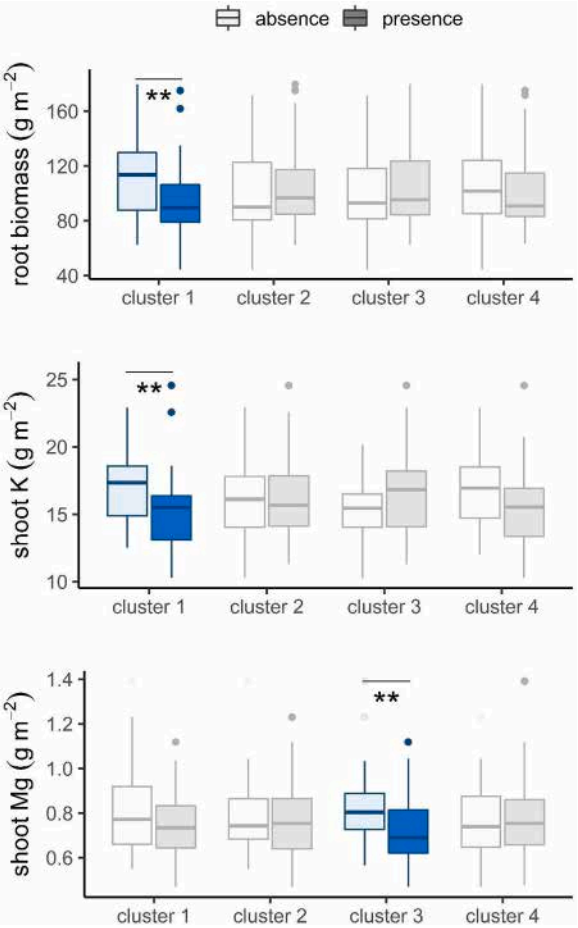

Fig. 4. Effect of the presence of the four functional groups (or clusters) in the mixtures on root biomass, shoot K content and shoot Mg content. Asterisks indicate significant differences between the mixtures where the cluster is present compared to those where the cluster is absent (*: $p < 0.05$, **, $p<0.01$, ***, $p<0.001$).

Levels of varietal/functional diversity

We cannot exclude that the level of varietal or functional diversity was insufficient for the expression of complementarity/facilitation and sampling/selection effects, although the experiment was especially designed to maximize intraspecific diversity. The initial panel included 57 varieties among 4 genetic types. Their functional traits showed a wide range of variation when compared to those of the subfamily *Pooideae*, notably for NO_3^- and NH_4^+ uptake and for root dry matter content (Cantarel et al., 2021), which are key traits for plant nutrient acquisition. Functional disparities between functional groups used in our experimental design were maximized on the basis of functional traits, including root traits that are known to be important drivers of ecological processes in the soil, partly through architecture for nutrient acquisition, and by stimulating various microbial communities through root exudates (Bardgett et al., 2014). Nevertheless, because plant traits are highly plastic and subject to strong environmental control (Hodge, 2004), it cannot be excluded that the root functional traits measured in mesocosms under greenhouse conditions differed from the trait values realized in the field, reducing the functional dissimilarities between varieties or functional groups. This may have ultimately led to a range of diversity that was insufficient to trigger functional effects.

Duration of the experiment

It has been shown in perennial ecosystems that the complementarity effect strengthens with time (Cardinale et al., 2007). For example, a time lag of 3 years was reported before detecting a significant effect of interspecific diversity on plant stoichiometry (Abbas et al., 2013), while a time lag of 4 years was necessary for the establishment of plot-specific microbial communities in the same experiment (Eisenhauer et al., 2010). This might be a reason for the absence of effects in our experiment, especially for the measurements of ^{15}N recovery that did not cover the entire vegetation cycle. ^{15}N recoveries measured in wheat biomass in this study are, however, consistent with the average of 38 % reported in the literature for ^{15}N recoveries after an addition of ^{15}N in cropping systems (Gardner & Drinkwater, 2009). In addition, nutrient concentration in wheat is highly dependent on inter-annual variability (Weih et al., 2016), so that one year may have been insufficient for detecting general patterns of intraspecific diversity effects on shoot nutrient content.

Biotic or abiotic stress conditions

Biotic stress as diseases can promote positive effects of diversity on biomass production in crop variety mixtures (Borg et al., 2018). Abiotic stress can also increase the frequency of positive interactions, which reinforces positive effects of diversity on ecosystem functioning (Holmgren & Scheffer, 2010; Maestre et al., 2009). Therefore, benefits of diversity could be lower under close-to-optimal conditions. No extreme climate event occurred during the year of our experiment, which may have constrained our ability to detect a diversity effect driven by positive interactions. In addition, 140 kg N ha^{-1} was applied in the field, so that the amount of N likely only partly limited wheat growth, which may have hidden potential complementarity or selection effects. The absence of diversity effects in our study may therefore be related to insufficient stressful conditions. Given the ongoing increase in extreme climate events, in particular in drought events (Seneviratne et al., 2021), and the need to reduce chemical N-fertilizers, intraspecific diversity effects may become stronger in the coming years.

Divergent effects of intraspecific diversity on shoot nutrient content

Contrary to our expectation of a positive effect of intraspecific diversity, mixtures with 2 varieties showed higher shoot Fe content than mixtures with 4 or 8 varieties, and higher shoot Zn content than mixtures with 8 varieties. In addition, the variance of plant NH_4^+ uptake had a negative effect of shoot Cu, Fe, Mg and Zn contents, which suggests a negative effect of plant functional disparities for this trait within a mixture. These results could emerge from a *negative selection effect* where the best performing variety or functional group in monoculture performs proportionally worse in mixture. This has already been empirically observed for interspecific diversity, when partitioning net diversity effect into complementarity effect and selection effect (Loreau & Hector, 2001) on biomass accumulation and its response to elevated CO_2 and N deposition (Reich et al., 2004), and for genotypic diversity effect on biomass production and plant abundance in species-poor costal ecosystem after warming (Reusch et al., 2005). Our experimental design did not allow to formally test this hypothesis, as it did not include replicates of the monocultures, and as it was not possible to distinguish individual varieties in the mixtures when the biomass was collected.

Given the complex interactions between plant nutrients and the greater or lesser control of the identity of varieties over these nutrients, various nutrients should be studied simultaneously in order to fully assess the effect of diversity on plant nutrition. Indeed, essential nutrients differ in their chemical properties, which determines their availability and mobility in the soil, as well as the corresponding plant assimilation pathway and metabolic functions, and in the end the nutrient influence on plant growth and development (Lambers & Oliveira, 2019). Moreover, multiple interactions between different nutrients affect the uptake, transport and assimilation of each other (Kumar et al., 2021). For example, an excess of N negatively correlates with P and Ca, and an excess of P negatively correlates with Zn, Cu and Fe in wheat (Pandey et al., 2020). These antagonistic interactions could explain why mixtures with 8 varieties had higher shoot Na content but a lower shoot Zn content than mixtures with 2 varieties in our study. Besides, nutrients are more or less controlled by the identity of the variety versus by environmental factors, which also indicates that potential complementarity or sampling effects emerging from varietal diversity are not likely to occur equally for each nutrient. For example, wheat Mg concentration has been shown to be mainly driven by the identity of the cultivated variety (Oury et al., 2006), which is also suggested by the significant negative effect of the presence of Cluster 3 on shoot Mg content observed in our study. Conversely, wheat Fe concentration (and to a lesser degree Zn concentration) depends mostly on environmental factors (Oury et al., 2006), which could explain why no identity effect of the functional groups was detected for these nutrients. Furthermore, nutrient concentration in the wheat biomass evolves differently between nutrients during life stages (Weih et al., 2016), suggesting that the effect of diversity may vary at a time among nutrients and among times for a given nutrient. This could be tested by studying the effect of diversity on multiple nutrients simultaneously and at several dates during the plant life cycle.

Conclusions

Our study reports different effects of the number of varieties and the number of functional groups on some wheat nutrients, but no effects on wheat biomass nor on N contents and fertilizer N recoveries in the plant-soil system. To our knowledge, this is the first time that the effect of varietal and functional diversity has been tested on multiple essential nutrients in addition to biomass and N in the plant-soil system. Our results therefore highlight the importance of considering different nutrients simultaneously, in order to capture their different behaviours in response to an increase in intraspecific diversity.

In our experiment, the varieties were blended in the mixtures to represent a gradient of variance in traits, and not in order to maximize complementarity and specific ecological processes, as we aimed to assess the effects of the number of varieties/functional groups, and not to identify the effects of specific mixtures. Thus, the absence of a detectable negative effect of diversity on biomass production or N content can be seen as an encouraging result. Indeed, if random mixtures behave on average as well as single varieties, a trait-based approach to intentionally design mixtures of varieties with the objective of maximizing complementarity could promote a positive effect of intraspecific diversity (Barot et al., 2017; Litrico & Violle, 2015). For instance, micronutrient deficiencies is a worldwide issue for human health, that can be reduced by biofortification using the considerable variability in the nutritional profiles of wheat varieties (Gupta et al., 2022; Pandey et al., 2016). However, some trade-off between micronutrients and between agronomical performance and nutritional traits sometimes makes this solution challenging, as for Fe and Zn deficiencies (Cabas-Lühmann et al., 2023; Fradgley et al., 2022; Xu et al., 2011). Wheat varieties mixtures designed on the basis of different nutrient profiles and agronomic performance could promote complementarity between varieties and multifunctionality of agroecosystems. Moreover, the potential of varietal crop mixtures should become particularly valuable in view of the increase in the frequency and severity of extreme climatic events such as drought and of the willingness of reducing fertilizers, that may enhance the positive effects of intraspecific diversity in the future.

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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

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.baae.2023.11.004.

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