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Guillaume Fried, Valérie Le Corre, Tiana Rakotoson, Julie Buchmann, Thomas Germain, et al.. Impact of new management practices on arable and field margin plant communities in sunflower, with an emphasis on the abundance of *Ambrosia artemisiifolia* (Asteraceae). *Weed Research*, 2022, 62 (2), pp.134-148. 10.1111/wre.12522 . hal-03605124

HAL Id: hal-03605124

<https://hal.science/hal-03605124>

Submitted on 10 Mar 2022

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Received: 25 September 2021 | Accepted: 17 December 2021

DOI: 10.1111/wre.12522

ORIGINAL ARTICLE

Weed Research | Wiley

Impact of new management practices on arable and field margin plant communities in sunflower, with an emphasis on the abundance of *Ambrosia artemisiifolia* (Asteraceae)

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Funding information: Office Français pour la Biodiversité

Subject Editor: Camilla Moonen, Scuola Superiore Sant'Anna di Pisa, Pisa, Italy

Abstract Some troublesome weeds such as *Ambrosia artemisiifolia* have led farmers to adopt herbicide-tolerant varieties (HTVs) in cultivated sunflower fields. Agricultural practices associated with the use of HTVs have raised concerns among public authorities, prompting the recommendation to monitor the potential effects of HTVs on biodiversity. In this context, we surveyed the vegetation of 239 sunflower fields and their margins in three French regions between 2017 and 2019, with a specific focus on *A. artemisiifolia*. We collected information on 21 explanatory variables covering agricultural practices, landscape factors, and spatio-temporal data. Herbicide-tolerant varieties fields were associated with lower weed diversity, but similar field margin community diversity. This lower weed diversity can be attributed to the greater use of herbicides and shorter crop rotations, whereas landscape factors may be more important for the diversity in field margins. Conventional fields with traditional varieties showed lower abundance of *A. artemisiifolia* compared with organic fields and HTV fields. *A. artemisiifolia* abundance was higher in the most infested region, in fields with a high proportion of sunflower crops in the crop rotation, late sowing dates, high numbers of hoeing operations and high numbers of post-emergence treatments. We interpreted the association of hoeing and HTV use with a high abundance of *A. artemisiifolia* as a response to weed infestation rather than its cause. In conclusion, no unintended effects of HTVs were found in field margins, but practices associated with HTVs lead to lower weed community diversity and HTV fields had still high *A. artemisiifolia* abundance after weed control.

Keywords: herbicide-tolerant varieties, weeds, invasive plant, common ragweed, biodiversity, agroecology, landscape.

Introduction

Sunflower (*Helianthus annuus* L.) was introduced from North America to Europe in the 16th century, but its large-scale cultivation in Europe mainly developed from the 1970s onwards to foster the independence of the European Community from the American oilseed monopoly. Previous studies have shown that following the increased acreage of sunflower crops and its more frequent return in crop succession since the 1970s, a specific and specialised weed flora has developed (Fried et al., 2009a). Among the species on the rise, *Ambrosia artemisiifolia* L. (common ragweed) is considered a particularly invasive and troublesome species in France (Chauvel et al., 2006) and also in some other countries (Croatia, Hungary, Slovenia, Turkey) (Kiss & Béres, 2006). In these areas, *A. artemisiifolia* spreads along roadsides and riverbanks, but its presence in agricultural fields can be very substantial. Due to its botanical proximity with sunflower (it belongs to the same Helianthae tribe, family Asteraceae), it remains difficult to control with the selective herbicides available for use on this crop (Pinke et al., 2011). To maintain effective chemical weed control solutions, herbicide-tolerant varieties (HTVs) obtained by mutagenesis were developed, marketed and quickly adopted by farmers in several European countries including Bulgaria, the Czech Republic, France, Hungary, Romania and Turkey (Pfenning et al., 2008). Two sunflower HTVs have been approved for use in France since 2010, one with tolerance to imazamox, the other one with tolerance to tribenuron. These two active ingredients have the same mode of action (inhibition of acetolactate synthase (ALS), HRAC Group 2); approximately 25% of the sunflower cultivation area grows a sunflower HTV in France (ANSES, 2020).

Given the expansion of HTVs over the past 10 years, and in response to public

concerns, the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) reviewed the use of these plant varieties. This review highlighted the risk of development of herbicide resistance in weed species, an increase in the use of herbicides compared with conventional crops (due to use of herbicides post-emergence) and, ultimately, the contamination of the environment with these active ingredients (ANSES, 2020). The risk factors are related to the management practices associated with HTV use, such as maintenance of short crop rotations and the application of herbicides with the same mode of action (ALS inhibitors) for weed control in sunflower and in other crops of the succession. One of the conclusions of this risk assessment was the need to set up an HTV monitoring programme to be able to detect changes in practices and their potential effects on weeds, the environment and human health (ANSES, 2020).

In the present study, we analysed—for the first time in Europe—weed communities associated with sunflower HTVs in comparison to traditional varieties (in both conventional and organic cropping systems) as well as potential unintended effects on the field margin vegetation. We sampled 239 sunflower fields in three regions (Cher, Côte-d'Or, Isère) for three years (2017, 2018, 2019). We addressed three main questions: First, what are the effects of region, landscape diversity and management practices on the diversity and composition of arable weed communities on the cropped areas of the field? Second, what are the effects of these same factors on the plant communities in the field margins? Third, what factors influence the abundance of *A. artemisiifolia* in cropped areas of the field and in the sterile strips of crop edges? Among management practices, we were particularly interested

in the effect of the practices specifically associated with the use of HTVs.

Materials and Methods

Study sites

Three regions were selected based on a gradient of residence time and current abundance level of *A. artemisiifolia* (Figure 1). Isère, located in the Rhône River basin, is the oldest region colonised by *A. artemisiifolia* (during the 19th century), where this species is also the most abundant (Chauvel et al., 2006). In contrast, *A. artemisiifolia* only became established in Côte-d'Or during the 1990s and this region is still little invaded (Chauvel et al., 2006). The Cher region occupies an intermediate position with the establishment of *A. artemisiifolia* in the second half of the 20th century and a strong increase in recent years. The status of sunflower in these regions is different: in the north (Cher, Côte d'Or), sunflower is one of the occasional summer crops inserted in the winter crop succession, but in Isère, sunflower is one of the main crops on which the crop rotation is based. In each of the three regions, professional agricultural organisations selected sunflower fields (Figure 1) from their network of farmers. Across the selected fields (239), we distinguished between three cropping systems: i) fields cultivated with sunflower HTVs (131 fields) and fields cultivated with traditional sunflower varieties, grown either using ii) conventional agricultural practices (95 fields) or iii) organic agricultural practices (13 fields).

Environmental and management variables

Farmers were interviewed to collect as much information as possible on their management practices. We ultimately obtained data for a total of 21 explanatory variables that were grouped into six

categories (Table 1). First, two *spatial variables* accounted for variations in soil and climatic conditions. Region (1) was used as a categorical variable (Cher, Côte-d'Or, Isère) summarising differences in edaphic and climatic conditions. Altitude (2) is a complex gradient incorporating abiotic conditions as well as topographic and landscape variations, opposing floodplains to more heterogeneous territories at the submontane level (Lososová et al., 2004). Second, *landscape variables* were estimated in two different ways. In both cases, the hypothesis was that a more diverse landscape offers more opportunities for diverse plant species to colonise the arable fields or the field margins (Gabriel et al., 2005). To characterise the landscape around the fields, we used the high nature value farmland indicator (*HNV* see Pointereau et al. (2010)) defined at the municipality scale, i.e. typically a few km² (3). High nature value relies on the calculation and combination of three components: i) crop diversity, ii) degree of intensification of farming practices (based on the level of pesticide use and the amount of fertilisation according to the French Agricultural Statistical Service) and iii) proportion of semi-natural habitats considered as beneficial to biodiversity. For the field margins, we also described the land use of the adjacent habitat based on the presence-absence of farm tracks (86 occurrences), paved roads (69), semi-natural habitats (including in particular ditches (17), hedges and small wood patches (36) or grasslands (18)), or croplands (138) (leading to four categorical variables) (4-7). In this case, the hypothesis was that field margins close to farm tracks between two croplands result in less diverse species richness due to a reduced species pool (mainly arable weeds) and more frequent management of the margin by farmers. Field margins near paved roads

may be less frequently managed and semi-natural habitats in the vicinity of the field margin (ditches, hedges, grasslands) may support higher diversity and more field-margin-specific species.

Five variables were used to describe the third category, namely *farming practices*. First, we computed the proportion of sunflower ($p_{Sunfl.}$) (8) and the proportion of summer crops ($p_{Sum.}$) (9) in the usual crop rotation of the surveyed fields. Tillage system (10) indicated the type of tillage usually used on the surveyed fields by distinguishing between conventional tillage (CT) with the use of a mouldboard plough and soil inversion versus minimum tillage (MT) with no ploughing and only tools for shallow tillage of the soil. Finally, we have taken into account the crop sowing date (11) and the quantity of nitrogen (kg/ha) supplied by mineral fertilisation ($N\ Fertil.$) (12).

The fourth category was *weed management practices* that were computed during the sunflower cropping season, the year of the floristic surveys. We used the number of hoeing passes on the inter-rows as a measure of mechanical weeding intensity (13). Then, we computed the herbicide treatment frequency index (TFI) which is the sum of the ratio between the applied dose and the approved dose ($TFI\ Herb.$) (14). This index gives an indication of the intensity of chemical weeding. We also recorded the number of pre-emergence (PRE) (15) and post-emergence (POST) (16) treatments. The three cropping systems: i) conventional systems using HTVs, ii) conventional systems using traditional varieties (Conv) and iii) organic systems (Org) were either used as a categorical variable in linear regression models (see below Data analysis) using the 239 fields (including 59 fields with no other variables on management), or as a supplementary variable for linear regression models using

the 180 fields with the detailed variables on farming and weed management practices.

For the field margins, we also added two more variables regarding the fifth category entitled *field margin management*: the field margin width (m) (17) and the margin management regarding the cutting practices (yes/no) (18). Finally, we accounted for three *temporal variables* which were encompassed in the sixth category. They included the date of each vegetation survey (continuous variable, Julian day from 1 January) (19), the average height of sunflowers at the time of the survey (20), as well as the year of the survey (qualitative variable, 2017, 2018 or 2019) (21) which can reflect differences in weather conditions.

Arable weed and field margin vegetation surveys

The surveys were carried out each year between 2017 and 2019 in June and July (between 4 June and 2 August). For sunflower, this corresponds to the period from stem elongation to inflorescence emergence. For the cropped area of the field, we sampled weeds over an area of 2000 m² (50 m x 40 m) located at least 20 m inside the field. The area was covered by two people walking along a W-shaped path and the species density was recorded according to the modified Barralis (1976) scale + : seen only once, 1: <1 ind/m², 2: 1-2 ind/m², 3-: 3-10 ind/m², 3+: 11-20 ind/m², 4: 21-50 ind/m², 5: > 50 ind/m².

In the field margins, we used the 500 ENI network sampling method (Fried et al., 2018): 10 quadrats of 1 m² (2 x 0.5 m) were arranged along the field margin with two sets of five contiguous quadrats separated by 30 m. The quadrats were placed in the centre of the field margin (i.e. equidistant from the field and the adjacent land cover). The average width of the field margins in our dataset was 2 m, ranging

from 0.5 m to 10 m. In each quadrat, the presence-absence of species was noted, giving each field margin a score of 1 to 10 for the species present.

Regarding *A. artemisiifolia*, we measured its density more precisely in the cropped areas of the field and in the inner crop edge of the field. In the cropped area of the field, we arranged 10 quadrats of 0.25 m² along the diagonal of the weed survey area of 50 m x 40 m. Along the crop edge, we counted the number of *A. artemisiifolia* in two 1 m² (2 m x 0.5 m) quadrats by positioning the quadrats used for field margin estimates on an area covering the cultivated field margin strip and the first inter-row of the crop.

Data analysis

For each observed species, we computed its regional frequency (number of occurrences/number of surveyed fields) and its local abundance (average abundance in fields where the species was present) in the cropped areas and the field margins. We also quantified the *fidelity* of species to the cropped area of the field using the Phi coefficient of association (Chytrý et al., 2002):

$$\text{Phi} = \frac{N \cdot n_p - n \cdot N_p}{\sqrt{n \cdot N_p \cdot (N - n) \cdot (N - N_p)}}, \text{ where } N \text{ is}$$

the total number of plots used (469 plots), N_p , the number of plots in arable habitats (239 cropped area plots), n , the number of occurrences of the species in the entire dataset (cropped area + field margin plots), and n_p , the number of occurrences of the species in the cropped area. Phi ranges from -1 (species characteristic of field margins) to +1 (species characteristic of cropped area). From this, a community weighted mean of the fidelity index ($\text{CWM}_{\text{fidelity}}$) was computed for each plot and was used as a supplementary variable to interpret the multivariate analyses. This index can reveal how much field margins are colonised by weeds from the cropped

area and conversely how much the cropped area is colonised by plants from the field margins (Metcalf et al., 2019).

Regarding management practices, we first assessed how the nine management practices (those numbered 8 to 16) differed between the three cropping systems. We used Kruskal-Wallis tests (followed by Dunn tests for post-hoc comparisons) for quantitative variables and Fisher's exact test for qualitative variables.

The diversity of plant communities was assessed with the first three orders of Hill numbers: ${}^qD = (\sum_{i=1}^S p_i^q)^{\frac{1}{1-q}}$, where S is the number of species in the assemblage, and the i^{th} species has relative abundance p_i . When $q=0$, 0D corresponds to species richness (S), as q tends to 1, 1D corresponds to the exponential of Shannon's diversity index ($\exp(H')$) and when $q=2$, 2D corresponds to the inverse of Simpson's index ($1/D$) (Chao et al., 2014). Increases in the parameter q of the Hill number give more weight to the presence of abundant species. Thus species richness (0D) is sensitive to rare species, whereas the inverse Simpson index (2D) is more sensitive to abundant species.

To test the effect of agro-environmental factors on Hill numbers measured in the cropped area, we used linear regressions with a stepwise selection (both backward and forward) of variables based on the Akaike information criterion to select the most parsimonious models. Continuous variables were standardised (mean=0, variance=1) prior to analysis. We inspected the normality of the residuals graphically with Q-Q plots and checked for the homogeneity of variance with Bartlett's test. Finally, we computed variance inflation factors (VIFs) to ensure that there was no issue of collinearity. When a qualitative variable was significant in a model, we used a post-hoc test to highlight significant differences between

levels/categories of that variable. For 59 fields, we had only information about the cropping system (Org, Conv, HTV), but no detailed data on management practices, which were only available for 180 fields. We therefore conducted a two-step analysis. For all 239 fields, we included only three explanatory variables: region (Cher, Côte-d'Or and Isère), landscape diversity (HNV) and cropping system (HTV, Conv or Org) as well as all the second-order interactions between these three variables. Then, for the 180 fields with detailed information on management practices, we carried out linear regressions, which included all variables except those specific to field margins (4-7, 17-18).

For the 180 fields with detailed information, the effect of the same agro-environmental variables on plant community composition was assessed using canonical correspondence analyses (CCA). A Monte-Carlo permutation test was performed to test the overall relationships between the environmental variables and the vegetation. Then we computed the gross and net effect of each variable. Gross effect corresponds to the inertia explained in a model with only the variable of interest. Net effect is the inertia explained by the variable of interest when partialling out the effect of the other variables with a partial CCA (pCCA). The same analyses (linear regressions on Hill numbers and CCA on community composition) were performed on field margin data, along with the additional six field-margin-specific variables (4-7, 17, 18).

Due to the high number of nul values (i.e. fields with no *A. artemisiifolia*), we modelled the abundance of *A. artemisiifolia* with a negative binomial regression, which can account for overdispersion. The response variable was the number of *A. artemisiifolia* individuals (in 10 (0.25 m²) quadrats) and the explanatory variables were the same as

those used for the analysis of community diversity and composition. All analyses were performed with R (Team, 2020) and the vegan package (Oksanen et al., 2020) for ordination analyses, or the MASS package (Venables & Ripley, 2002) for negative binomial generalised linear models.

Results

A total of 439 plant species was observed, with 243 arable weed species identified in the cropped area of the 239 surveyed fields and 398 plant taxa observed in the 225 field margins. The cropped areas and the field margins shared 202 species (46%), 41 were only found in cropped areas (9.3%) and 196 species were only found in field margins (44.7%).

In the 2000 m² of cropped area, species richness varied from 2 to 40 species with a mean of 13.3 species. The five most frequent species were *Fallopia convolvulus* (68%), *Senecio vulgaris* (54%), *Convolvulus arvensis* (54%), *A. artemisiifolia* (54%) and *Chenopodium album* (44%). The five species with the highest fidelity to the cropped area were *F. convolvulus* (0.42), *S. vulgaris* (0.37), *Persicaria maculosa* (0.29), *Solanum nigrum* (0.25) and *Echinochloa crus-galli* (0.25).

In field margins, species richness was higher than in the cropped area with 8 to 50 species and an average of 23.2 species within only 10 m². The five most frequent species were *C. arvensis* (83%), *Dactylis glomerata* (69%), *Elytrigia repens* (69%), *Lolium perenne* (67%) and *Arrhenatherum elatius* (61%). The species with the highest fidelity to field margins were *D. glomerata* (-0.71), *A. elatius* (-0.67), *Anisantha sterilis* (-0.58), *E. repens* (-0.56) and *Galium album* (-0.55). Table S1 gives the full list of the 439 plant taxa observed in the cropped areas and in the field margins with their regional frequency, local abundance and fidelity index to

cropped areas. There was a slight positive correlation between species richness in the cropped areas and in the field margin (Pearson's $r=0.181$, $P=0.007$, Figure 2a).

Overview of the three cropping systems

In the 180 selected fields with detailed information, the management practices in the three cropping systems differed significantly in many ways (Table 2). Organic cropping systems are by definition characterised by the absence of chemical weeding and mineral fertilisation. In these systems, the number of hoeing passes was higher and the sowing dates were later, but the proportion of sunflower or summer crops did not differ from the two other cropping systems. The HTV cropping system had a higher herbicide TFI than conventional systems, a higher proportion of sunflower and summer crops in the crop succession, but a lower level of mineral fertilisation. As expected, the number of pre-emergence treatments in HTV fields was lower than in conventional fields, in favour of a higher number of post-emergence treatments. Among HTVs, 38 used imazamox, 32 tribenuron, and 28 used HTVs without the associated herbicide. The tillage systems (CT versus MT) did not differ between the three cropping systems.

Arable weed communities in the cropped areas

In the cropped areas, species diversity was predominantly explained by the cropping system (Table 3a). Species richness was higher in organic fields than in conventional or HTV fields (Figure 2b). Although the range of species richness was similar between conventional and HTV fields, the violin plots (Figure 2b) showed that many HTV fields had low species richness, i.e. around 10 species. The exponential Shannon index was higher in organic and conventional fields than in HTV

fields, but there were no differences between organic and conventional fields (Figure S1). There was also a significant interaction between landscape diversity and cropping system (Table 3a), with a positive effect of landscape diversity only observed in organic fields (Figure 2c). Finally, the inverse Simpson index was higher in conventional than in HTV fields, with no other significant differences (Figure S1). Again the interaction effect indicated the positive effect of landscape only observed in organic fields.

For the 180 fields with detailed information, the selection procedure kept four explanatory variables for species richness in the reduced model including, in decreasing order of explained variance, herbicide TFI, % of summer crops in the crop succession, altitude and number of post-emergence treatments (Figure 3a). Herbicide TFI and proportion of summer crops in the rotation were negatively correlated, whereas altitude was positively correlated with species richness (Fig. 3b). Four variables were selected for the model explaining Shannon diversity, with herbicide TFI accounting for the largest proportion of explained variance (Fig. 3c). Shannon diversity was lower in fields with high herbicide TFI and in fields under minimum tillage (Fig. 3d). Five variables were selected for the inverse Simpson index which was mainly explained by region, tillage system and herbicide TFI (Fig. 3e). Again, high herbicide TFI and minimum tillage systems were associated with reduced inverse Simpson diversity (Fig. 3e).

The canonical correspondence analysis (CCA) showed that there was a significant correlation between agro-environmental variables and weed community composition ($F= 2.01$, $P<0.001$). The 15 explanatory variables explained 17.7% of the inertia. Region and year had the highest net effect (Table 4),

followed by the number of pre-emergent treatments and altitude. The first CCA axis (20.5% of inertia explained) separated fields according to region as well as to crop rotation expressed by means of the variables proportion of sunflower and summer crops (Figure 4). Fields in the Côte-d'Or and Cher regions with low proportions of summer crops showed negative loadings, whereas fields in the Isère region with high proportions of summer crops showed positive loadings. This axis was also related to survey year—with 2017 at the negative loadings end of the axis in contrast to 2018 and 2019—, survey date and sunflower height, the latter two variables being negatively correlated with CCA Axis 1. *Euphorbia exigua*, *Cyanus segetum* and *Lapsana communis* were characteristic weeds of sunflower fields with high proportions of winter-sown crops in the rotation in the Cher and Côte-d'Or regions, whereas *A. artemisiifolia*, *Amaranthus retroflexus* and *E. crus-galli* were associated with sunflower fields in Isère with a higher proportion of summer crops in the rotation. The second axis (11.2% of inertia explained) separated fields according to management practices and more specifically mechanical versus chemical weed management. Minimum tillage and high number of hoeing passes were distinct from high number of pre-emergence treatments and overall use of herbicides (TFI Herb.). Species such as *Rumex* spp. and *Artemisia vulgaris* were associated with hoeing and low herbicide TFI, whereas *Bidens tripartita* and *Persicaria lapathifolia* were associated with a high number of pre-emergent treatments at the end of the herbicide use intensity gradient. When using cropping system as a supplementary variable, CCA Axis 1 discriminated the HTV system (higher values) from the conventional system (lower values) (Kruskal-Wallis chi-squared = 13.227, $P=0.001$), and the CCA

Axis 2 discriminated the organic system (lower values) from the two other cropping systems (Kruskal-Wallis chi-squared = 32.162, $P<0.001$). Weed community species richness was negatively correlated with both CCA axes 1 (Pearson's $r=-0.174$, $P=0.020$) and 2 (Pearson's $r=-0.350$, $P<0.001$). The CWM of fidelity to arable field (i.e. species more characteristic of the cropped areas) was positively correlated with CCA Axis 1 (Pearson's $r=0.223$, $P=0.003$).

Field margin plant communities

The diversity of field margin plant communities was mainly driven by landscape diversity and region (Table 3b). The three Hill numbers increased with landscape diversity (HNV) and differed across the three regions (Figure 2d). Species richness was higher in field margins of Isère than in Côte d'Or (Figure 2d). The violin plot indicated that there was only one peak of values in Côte-d'Or around 20 species per field margin, whereas in both Cher and Isère, the violin plots indicated a higher first peak of values (20-25) and a second peak with more than 30 species per field margin. The exponential Shannon index and the inverse Simpson index were lower in Côte d'Or than in the Cher and Isère regions (Figure S1).

For the 168 field margins with detailed information, six variables were selected for the reduced model explaining species richness and eight variables for the reduced models of the Shannon diversity index and the inverse Simpson index (Fig. 5). For each model, region and presence/absence of semi-natural in the vicinity of the field margins explained the highest proportion of variance (Fig. 5). All three Hill numbers were higher in Isère region, particularly where field margins were adjacent to semi-natural habitats (hedges, ditches, grasslands), whereas they decreased with the proportion of

sunflower crops in the crop rotation (Fig. 5). The Shannon diversity index and the inverse Simpson index also decreased with the date of the survey (Figure 5b, c).

The CCA for the field margins showed a significant relationship between plant community composition and agro-ecological variables ($F=1.392$, $P<0.001$). The 19 explanatory variables explained 17.8% of the inertia of the species abundance matrix (Table 4). On the first CCA axis (11.4% of variation), field margins with *Poa annua*, *Polygonum aviculare*, as well as *Plantago major* and *Taraxacum officinale*, next to farm tracks and managed by farmers were well separated from wider margins, next to paved roads and near semi-natural habitats (grasslands, ditches, hedges) in more diversified landscapes (high HNV values) that typically included species such as *A. elatius*, *Festuca rubra*, *Ranunculus repens*, or *Rumex acetosa* (Figure 6). Axis 1 of the CCA was also negatively associated with N fertilisation and to some extent, it contrasted the Côte-d'Or and Cher regions. The first CCA axis was negatively correlated with the CWM of arable field fidelity index (Pearson's $r=-0.283$, $P<0.001$). The second axis (10.5% of inertia) separated the Isère region, with species such as *A. artemisiifolia*, *Cynodon dactylon* or *Holcus lanatus* on the positive loadings, from the Côte d'Or and Cher regions, with species such as *Lysimachia arvensis*, *Alopecurus myosuroides* or *Heracleum sphondylium* on the negative loadings. Positive coordinates were also associated with high landscape diversity (HNV), high proportion of summer-sown crops, no cutting of the field margin, higher altitude, hoeing, whereas negative coordinates were associated with margins with higher amount of N fertilisation. This second CCA axis was positively correlated with species richness (Pearson's $r=0.209$, $P=0.006$) and with the CWM of arable field fidelity index (Pearson's $r=0.153$, $P=0.047$).

Cropping systems were not discriminated along Axis 1 (Kruskal-Wallis chi-squared = 1.384, $P=0.501$), but field margins of organic fields had significantly higher values on CCA Axis 2 than the two other cropping systems (Kruskal-Wallis chi-squared = 20.82, $P<0.001$).

Factors influencing the abundance of Ambrosia artemisiifolia L.

Ambrosia artemisiifolia was recorded in 121 out of the 239 surveyed fields (51%) and in 36 (15%), it occurred only at the edges of the crops. Its maximum density was 404 individuals/m² in cropped areas and 335 individuals/m² in crop edges. However, the average density was higher in the crop edges (mean=17.7±43.9) than in cropped areas (7.5±36.8, Wilcoxon paired test, $P<0.001$) and there was a positive correlation between the *A. artemisiifolia* density in both areas (Pearson's $r=0.55$, $P<0.001$). *Ambrosia artemisiifolia* abundance differed among the three cropping systems in the cropped areas (Kruskal-Wallis test, chi-squared = 22.29, $df = 2$, $P < 0.001$) and the crop edges (Kruskal-Wallis test, chi-squared = 11.11, $df = 2$, $P = 0.004$). The densities were lowest in conventional cropped areas (median=0, range=0-12, mean =0.77 ind/m²) compared with both organic (median=1, range=0-95.6, mean =13.5 ind/m²) and HTV cropped areas (median=0, range=0-404, mean =11.9 ind/m²). Differences were similar in the crop edges, with the lowest values for conventional crop edges (median=0, range=0-335, mean =13.9 ind/m²) relative to organic (median=4, range=0-56, mean =13.6 ind/m²) and HTV crop edges (median=1, range=0-257.5, mean =20.8 ind/m²).

The negative binomial regression showed that a high number of *A. artemisiifolia* individuals in cropped areas was positively associated with the Isère region, late sowing date, high proportion of

sunflower crops in the crop succession, the number of post-emergent treatments as well as high number of hoeing passes. Low *A. artemisiifolia* density was associated with the Côte-d'Or region, high proportion of summer-sown crops and high level of herbicide TFI (Table 5). Results were similar for the crop edge, except that there was no effect of sowing date and hoeing passes (data not shown).

Discussion

The aim of this study was to assess the effect of different cropping systems and new management practices on arable and field margin plant communities as well as on the abundance of *A. artemisiifolia*, a troublesome invasive weed, with a particular attention on the use of sunflower HTVs. Only two previous studies have attempted to identify the drivers of *A. artemisiifolia* abundance in arable fields (Pinke et al., 2011, Pinke et al., 2019), and HTV use has to date only been assessed in terms of control efficiency for a few major weeds in Bulgaria (Tonev et al., 2020).

We found that weed communities in the cropped area of the cultivated field depended more directly on farming and weed management practices relative to field margin plant communities, which in turn were more influenced by landscape diversity and spatial variables (region, altitude) as shown in previous studies (Solé-Senan et al., 2014).

Effect of spatial variables

Differences between the three regions were expected because they represent distinct soil and climatic conditions and, in part, distinct species pools. Fields in Isère were characterised by the presence of more thermophilous or sub-Mediterranean species that reach the north of the Rhône Valley (*Avena barbata*, *C. dactylon*, *Reseda phyteuma*, *Sorghum*

halepense), but that are not or hardly present in Cher and Côte-d'Or, two regions with a relatively more medio-European vegetation. There are also some differences in soil properties, with mainly silty clay soils with neutral to basic pH in Cher and Côte-d'Or, contrasting with Isère where soils are generally sandy-silty and more acidic. Finally, we found that altitude was correlated with higher weed species diversity. Several studies have already reported increased weed species richness with elevation, which is usually interpreted as an effect of lower agricultural intensity in upland areas (Lososová et al., 2004, Fried et al., 2008).

Effect of landscape

A diversified landscape around the surveyed field was associated with increased diversity particularly in field margins, as already highlighted in several studies (Gabriel et al., 2005, Gaba et al., 2010). In cropped areas, the positive effect of the landscape was only observed in organic fields, perhaps suggesting that the individuals of casual weed species, spreading from the neighbouring habitats and potentially increasing the species diversity in the cropped area (Metcalf et al., 2019), are removed by more intensive management practices in conventional and HTV sunflower fields. In field margins, the type of land use adjacent to the field margin had a stronger influence than landscape diversity at a larger scale. The presence of ditches, hedges, small woods or grasslands close to the field margin was found to be a clear and strong factor associated with greater species diversity (Blaix & Moonen, 2020), together with less weedy species and more species specific to field margins (Fried et al., 2018, Cirujeda et al., 2019). Neighbouring infrastructures also influenced the management of the field margins cut more often near farm tracks than near paved roads. Our study

highlighted a gradient from poor and highly disturbed field margins dominated by weedy species (*Poa annua*, *Polygonum aviculare*) near farm tracks between fields to more diverse field margins dominated by grassland species (*A. elatius*, *Festuca rubra*) near paved roads (usually not managed by farmers) and semi-natural habitats, confirming the classification of field margins proposed by Cirujeda et al. (2019) for Mediterranean agroecosystems.

Effect of farming and weed management practices

Herbicide use intensity accounted for a very large part (86%) of the explained variation in cropped area species richness, whereas it only explained very little (8%) of the composition observed in arable weed communities. Herbicide use intensity was associated with a reduction in species richness, as well as a reduction in the exponential Shannon index and the inverse Simpson index. Although this relationship was expected in a post-herbicide weed survey like the one presented in this study, this relationship can sometimes be blurred by the fact that farmers adapt their herbicide use intensity to the initial weed infestation (Colbach et al., 2020).

Farming practices such as crop rotation also influenced the number and the taxonomic identity of arable weeds. Sunflower fields that were in a crop succession with a high proportion of other summer-sown crops had lower species richness. This result is consistent with other studies that have highlighted the positive influence of both crop rotation diversity (Murphy et al., 2006, Ulber et al., 2009) and crop sowing date diversity (Mahaut et al., 2019) on arable weed community richness. As shown on the first CCA gradient (Axis 1, Figure 4), sunflower fields included in summer rotations had a pool of summer-germinating weed species (*A. retroflexus*, *E. crus-galli*), whereas sunflower fields with

a significant proportion of winter-sown crops harboured additional species usually occurring in wheat or oilseed rape (e.g. *A. myosuroides*, *C. segetum*, *Geranium dissectum*).

Factors associated with Ambrosia artemisiifolia abundance

As also observed in Hungary, the abundance of *A. artemisiifolia* was significantly higher at crop edges than in the cropped areas of the fields (Pinke et al., 2011). Chemical weed management operations are often less efficiently applied near the edges of crop fields and the light conditions of crop edges are also usually more favourable to arable weeds (Kleijn & Van Der Voort, 1997, Fried et al., 2009b, Solé-Senan et al., 2014).

As expected, the abundance of *A. artemisiifolia* depended strongly on the region, being higher in the Isère region than in the Côte-d'Or region (with the Cher region as the reference). This result is consistent with the fact that, in Isère, *A. artemisiifolia* has occupied the landscape (roadside, riverbank) for several decades, but in Côte-d'Or, its invasion is relatively recent (about 25 years; Chauvel et al. 2006). Differences in soil properties between the regions are not thought to drive *A. artemisiifolia* abundance: a previous study highlighted that its abundance in France was independent of soil characteristics (Fumanal et al., 2008).

Most interestingly, there are farming and weed management factors associated with *A. artemisiifolia* abundance. High proportions of sunflower crops in a crop succession favoured *A. artemisiifolia*. This was expected because controlling *A. artemisiifolia* in sunflower crops is a challenge for farmers and, compared with other crops, *A. artemisiifolia* densities are always higher in sunflower (Pinke et al., 2011). More surprisingly, a high proportion of summer-

sown crops was associated with lower *A. artemisiifolia* abundance. This may be due to the higher effectiveness of weed management options available in some summer crops, such as maize or sorghum.

Relationships between weed management variables and *A. artemisiifolia* abundance are more complex to interpret due to the fact that we had only one census for measuring *A. artemisiifolia* abundance and given that farmers adapt certain practices according to the level of *A. artemisiifolia* (Colbach et al., 2020). On the one hand, the negative relationships between herbicide use intensity and *A. artemisiifolia* densities can be interpreted as intensive chemical weeding that created a significant filter for *A. artemisiifolia*. On the other hand, it may be inconsistent to interpret the positive relationships between the number of post-emergence treatments or hoeing passes and abundance of *A. artemisiifolia* as evidence that these practices favour *A. artemisiifolia*. Instead, farmers increase the number of hoeing passes (for organic and conventional farmers) or apply imazamox or triburon herbicides in post-emergent treatments when they observe high densities of *A. artemisiifolia*. Because our surveys in the cropped areas of fields were performed after the time of application of post-emergence herbicide treatments, the positive relationship between these treatments and the presence of *A. artemisiifolia* suggests limited efficacy when infestation levels are high.

Non-intended effects of herbicide-tolerant varieties

A limitation of our study is the small number of organic fields and their concentration in the Isère region. This reflects the reality on the ground, because there are few organic sunflower crops in the northern part of France. Conclusions

associated with organic systems must therefore be taken with caution and a larger sampling of these systems is needed for future studies. Some patterns associated with cropping systems may be region specific. In a recent study, lower abundances of *A. artemisiifolia* in organic fields in Austria compared with organic fields in Hungary were interpreted as a longer tradition of organic farming in Austria and better expertise in non-chemical weed control techniques that are sometimes more effective than herbicides (Pinke et al., 2019). However, this does not affect one of our main results, which showed that HTV fields were associated with lower arable weed community diversity than organic and conventional fields. In contrast, no significant effects of HTVs could be identified on the diversity of field margin plant communities that depended much more on abiotic and landscape factors. Our study design did not allow us to compare the efficacy on *A. artemisiifolia* control, but there was evidence that conventional fields had lower abundance levels of *A. artemisiifolia* than organic or HTV fields.

As expected by the ANSES risk analysis (ANSES, 2020), HTVs were associated with higher herbicide TFI and higher proportion of sunflower crops in the crop rotation that can partly explain the observed patterns. The fact that a high herbicide TFI reduced arable weed species richness and that the number of post-emergence treatments tended to be associated with a lower inverse Simpson index (i.e. number of abundant species) can explain the lower weed diversity observed in HTV fields. Finally, a high proportion of sunflower crops was associated with lower diversity in field margin plant communities. Although we did not detect an effect of HTVs on field margin plant diversity, if HTVs rely on short crop rotations with a high proportion of sunflower crops, it may

slightly reduce the diversity of the species pool in the field margin. Taken together, these results suggest that the use of HTVs can have indirect effects, mainly on arable weed communities, through associated management practices, e.g. higher use of herbicides, post-emergence treatments and reduced crop rotation diversity. It should be pointed out that the present study took place less than 10 years after the first HTVs were approved for use. Some unintended effects that have not yet been detected may occur in the future, especially because, in some of the surveyed fields, farmers had only recently adopted this new practice. The use of compounds from the HRAC 2 group also poses the important risk of selecting for resistant species, which is already the case for *A. artemisiifolia* in France (Loubet et al., 2021). Several resistance situations have been detected to date, although still involving low proportions of resistant plants. No situations have been identified in our field network that can be confidently assimilated with resistance development (analysis in progress - data not shown). The presence of herbicide-tolerant sunflower volunteers in the following crops of the rotation can also be an agronomic problem. Their management in soybean crops where the same herbicides of the HRAC 2 group are used should require particular consideration, especially in reduced tillage systems where volunteers are more frequent. A second monitoring study in 5 to 10 years may be useful, because changes in weed communities have been shown to occur at the scale of two to three decades (Fried et al., 2009a).

Acknowledgements: This study was supported by the *Office Français pour la Biodiversité* (OFB) through the Ecophyto program ENI-VTH. We thank the 112 farmers for allowing us to survey the weeds in their fields and for providing information

on their farming practices, as well as Laetitia Masson (*Chambre agriculture de l'Isère*), Christophe Benas (*Chambre agriculture de Côte d'Or*), Amandine Guimard and Benjamin Pointereau (the farmers' association FDGEDA du Cher) and the involved participating professional organisations (*Chambre agriculture de l'Isère, Chambre agriculture de Côte d'Or* and FDGEDA du Cher). We thank Carolyn Engel-Gautier for improving the English language.

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Table 1. Summary of the 21 explanatory variables used, grouped in six broad categories.

Category	Name of the variable with its abbreviation and number				
Spatial	(1) Region : Cher (CH), Côte-d'Or (CO) and Isère (IS)	(2) Altitude (Alt)			
Landscape	(3) High nature value indicator (HNV)	(4-7) Field margin land use: presence-absence of farm track, paved road, semi-natural habitats and cropland			
Farming practices	(8) proportion of sunflower crops in crop rotation (p_Sunfl)	(9) proportion of summer crops in crop rotation (p_Sum)	(10) Tillage system: conventional tillage (CT), minimum tillage (MT)	(11) Crop sowing date	(12) Amount of N fertilisation
Weed management practices	(13) Number of hoeing passes (Hoeing)	(14) TFI Herbicides (TFI Herb) = ratio applied dose to approved (standard) dose	(15) number of pre-emergence treatments (PRE)	(16) number of post- emergence treatments (POST)	
Field margin management	(17) Margin width	(18) Margin management			
Temporal	(19) Date of the weed survey	(20) Sunflower height (Sunfl height)	(21) Year of the weed survey		

Table 2. Comparison of management practices across the three cropping systems. Mean and standard deviation of continuous variables are given for the three cropping systems: organic, conventional and herbicide-tolerant varieties (HTVs). Different letters indicate significant differences based on a post-hoc Dunn test. Counts are given for the qualitative variable. Units of the variables are given between brackets. Abbreviations of variable names are given in Table 1.

Continuous variables	Organic (n=12)	Conventional (n=70)	HTV (n=98)	Kruskal-Wallis test
p_Sunfl [0-1]	0.23±0.10 ^{ab}	0.24±0.09 ^a	0.28±0.09 ^b	<i>P</i> =0.010
p_Sum [0-1]	0.36±0.15 ^{ab}	0.30±0.13 ^a	0.40±0.18 ^b	<i>P</i> <0.001
Sowing date [Julian day]	119.1±15.6 ^b	104.0±12.0 ^a	109.1±12.3 ^b	<i>P</i> <0.001
TFI Herb	0.00±0.00 ^a	1.24±0.50 ^b	1.48±0.52 ^c	<i>P</i> <0.001
PRE [number of passes]	0.00±0.00 ^a	1.94±0.78 ^b	1.09±0.61 ^a	<i>P</i> <0.001
POST [number of passes]	0.00±0.00 ^a	0.13±0.45 ^a	0.72±0.47 ^b	<i>P</i> <0.001
Hoeing [number of passes]	2.00±0.74 ^b	0.31±0.67 ^a	0.53±0.79 ^a	<i>P</i> <0.001
N fertilisation [kg.ha ⁻¹]	0.00±0.00 ^a	330.1±837.9 ^b	116.8±397.0 ^a	<i>P</i> =0.010
Qualitative variable				Fisher test
Tillage system				<i>P</i> =0.456
CT	8	55	79	
MT	4	15	19	

Table 3. Summary table of the reduced models explaining variations in species richness (S), the exponential of Shannon’s diversity index (exp (H’)), and the inverse Simpson index (1/D), for the cropped areas (a) and for the field margins (b). HNV: High nature value farmland indicator. Significant factors are shown in bold. ‘-’ indicates that the factor had not been retained in the reduced model.

a) Cropped areas		S (Adj. R²: 0.083)			exp (H') (Adj. R²: 0.058)			1/D (Adj. R²: 0.055)		
Variables	df	F-value	P-Value	df	F-value	P-Value	df	F-value	P-Value	
HNV	1	1.72	0.191	1	0.18	0.673	1	0.02	0.901	
Region	2	0.91	0.404	-	-	-	-	-	-	
Cropping system	2	11.31	<0.001	2	5.11	0.007	2	4.32	0.014	
HNV x Cropping system	-	-	-	2	4.08	0.018	2	4.58	0.011	
Residuals	227			227			227			
b) Field margins		S (Adj. R²: 0.089)			exp (H') (Adj. R²: 0.058)			1/D (Adj. R²: 0.055)		
Variables	df	F-value	P-Value	df	F-value	P-Value	dDf	F-value	P-Value	
HNV	1	10.8	0.001	1	10.7	0.001	1	9.70	0.002	
Region	2	5.23	0.006	2	5.39	0.005	2	5.62	0.004	
HNV x Region	2	2.53	0.082	2	2.80	0.063	2	2.50	0.084	
Residuals	213			213			213			

Table 5. Summary of the negative binomial regression explaining the number of *Ambrosia artemisiifolia* individuals in sunflower fields. Bold figures indicate variables that have a significant effect.

Variables	Estimate	Std. Err.	z value	P-value
<i>Temporal variables</i>				
Year2018	-0.99	0.66	-1.51	0.131
Year2019	0.33	0.66	0.50	0.619
Survey_date	-0.42	0.45	-0.93	0.352
<i>Region</i>				
Côte-d’Or	-4.58	1.11	-4.14	<0.001
Isère	3.60	0.79	4.56	<0.001
<i>Landscape</i>				
Alt	-0.40	0.28	-1.41	0.159
HNV	0.05	0.22	0.21	0.830
<i>Farming practices</i>				
MT	-1.00	0.62	-1.62	0.106
Sowing date	0.81	0.26	3.09	0.002
Sunfl height	0.31	0.37	0.83	0.408
p_Sum	-0.72	0.25	-2.85	0.004
p_Sunfl	0.52	0.24	2.14	0.033
N Fertilisation	0.34	0.25	1.34	0.180
<i>Weed management practices</i>				
TFI Herb	-0.82	0.32	-2.57	0.010
PRE	0.45	0.39	1.16	0.246
POST	2.73	0.31	8.88	<0.001
Hoeing	0.74	0.25	2.92	0.004

For the definition of variables, see Table 1.

Figure 1. Map of the surveyed fields (circles) in the three regions. Red circles are those for which the interview with the farmer was comprehensive (n=180), green circles are those for which only minimal information on management practices was available (n=59).

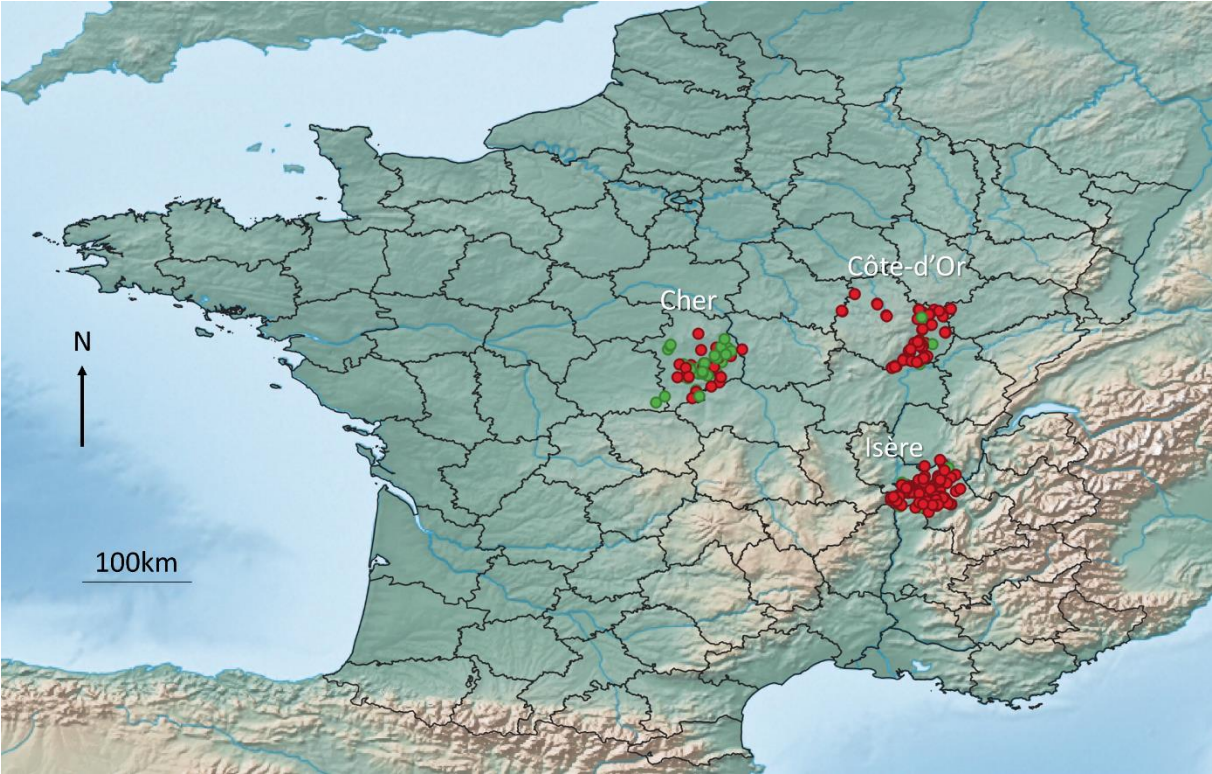


Figure 2. a) Relationship between species richness in the cropped areas and in the field margins (Pearson's $r=0.181$, $P=0.007$), b) Species richness of the cropped areas in the three cropping systems, c) Predicted values of the exponential of Shannon's diversity index ($\exp(H')$) according to landscape diversity (HNV) for the three cropping systems (conventional (Conv); Herbicide-resistant varieties (HTVs); organic (Org)), d) Species richness of the field margins in the three regions (CO: Côte-d'Or, CH: Cher, IS: Isère). In b) and d), the boxes represent 50% of the values around the median (horizontal line) and the extremities of the vertical lines represent the minimum and maximum values. The violin plots show the density of the data at each value. Different letters indicate significant differences based on post-hoc tests.

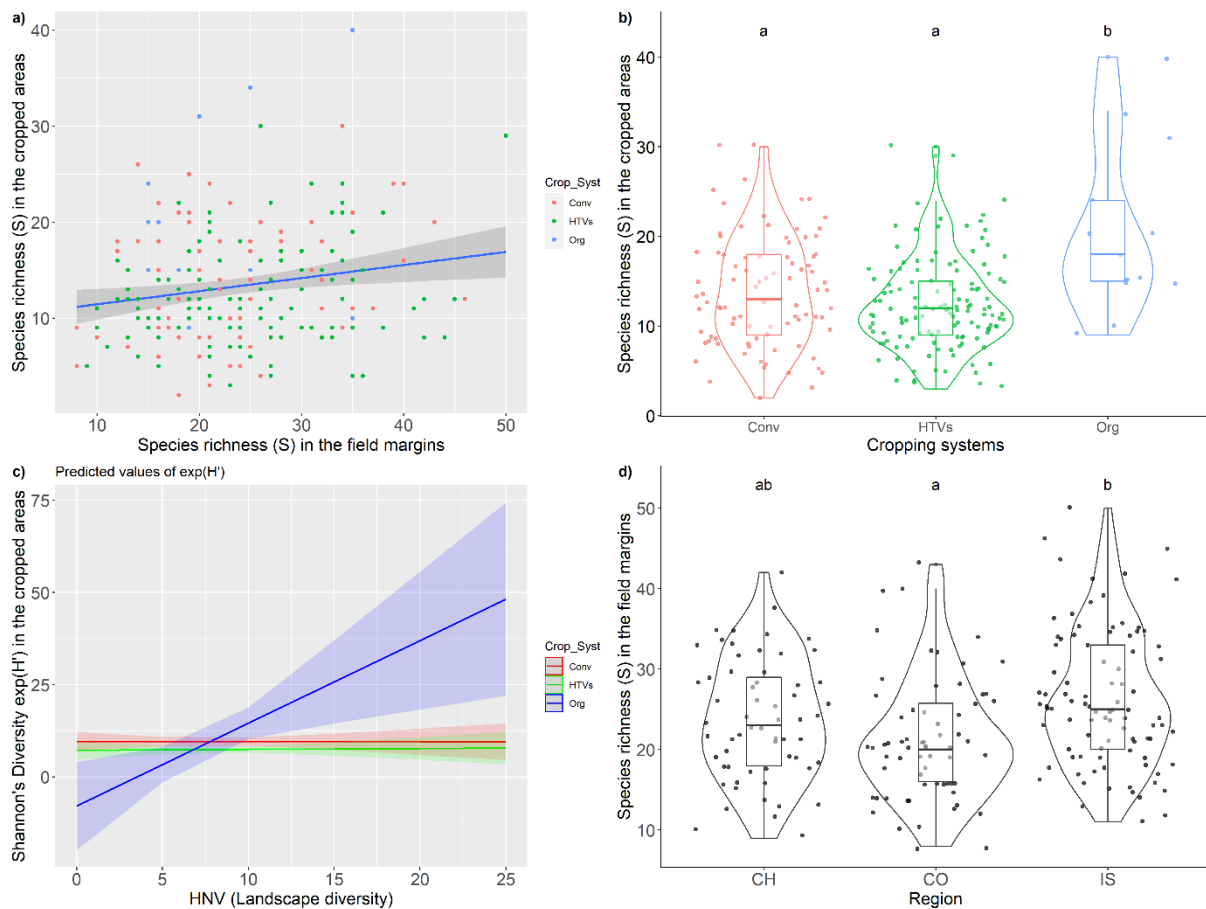


Figure 3. Percentage of explained variation for variables selected in the reduced models (left) and standardised effects of the fixed variables in the reduced model (right) for species richness (a,b), the exponential of Shannon’s diversity index (c,d) and the inverse Simpson index (e, f) of arable weed communities in the 180 cropped areas with detailed agro-environmental information. Variable definitions are given in Table 1.

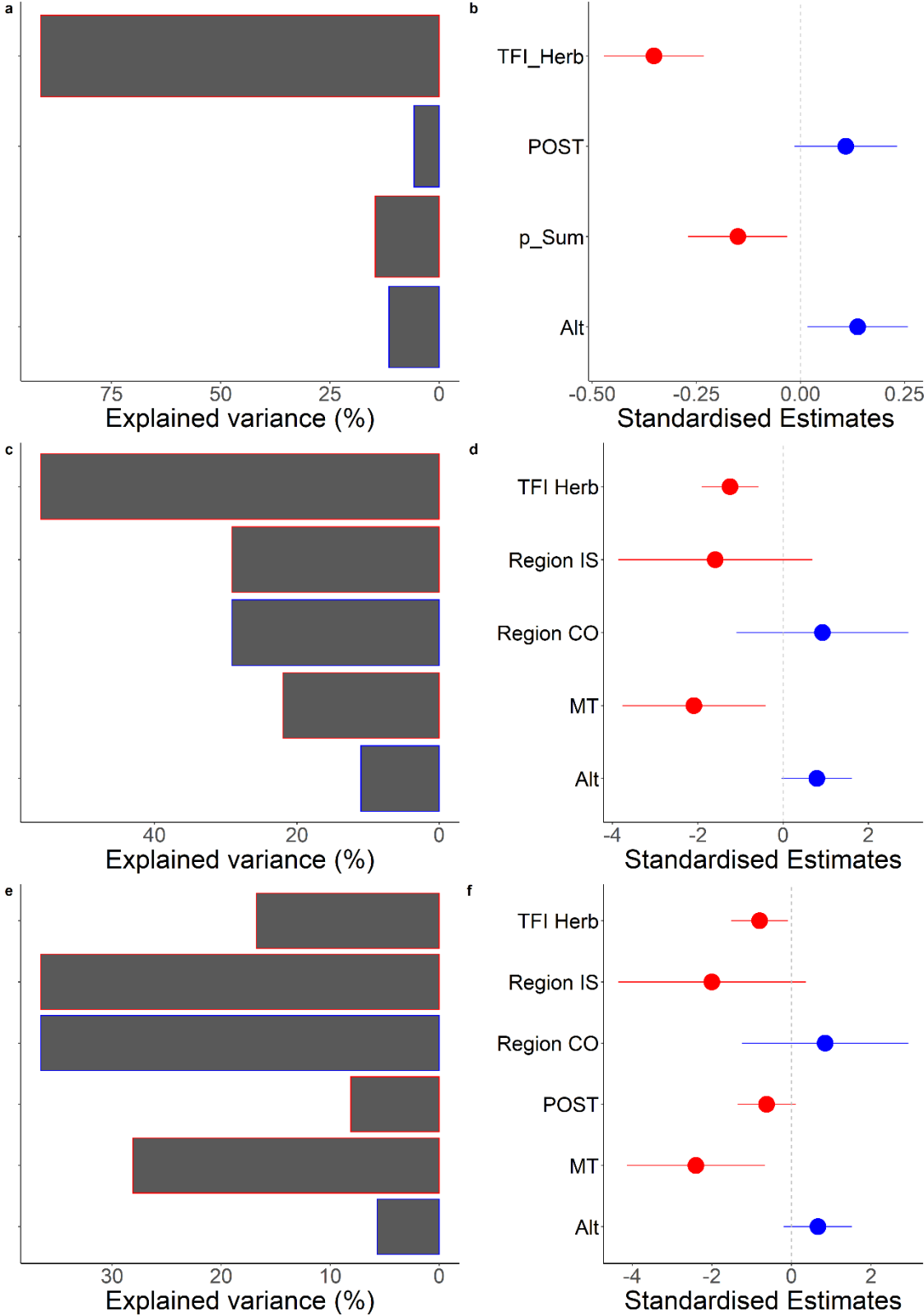


Figure 4. Ordination diagrams of the canonical correspondence analysis (CCA) model on cropped areas containing a) the sites and the explanatory variables and b) the species (coded with the first four letters of the genus and species names). Colours indicate the three cropping systems (red: conventional, green: use of herbicide-resistant varieties, blue: organic) and shapes, the three regions (circles: Cher, triangles: Côte-d’Or, squares: Isère). The abbreviations used for the variables are defined in Table 1. The dashed vector represents the supplementary variable $CWM_{Fidelity}$ directed towards communities dominated by arable habitat specialists. The species names are only displayed for the species with the highest fit (other species are indicated by a grey circle).

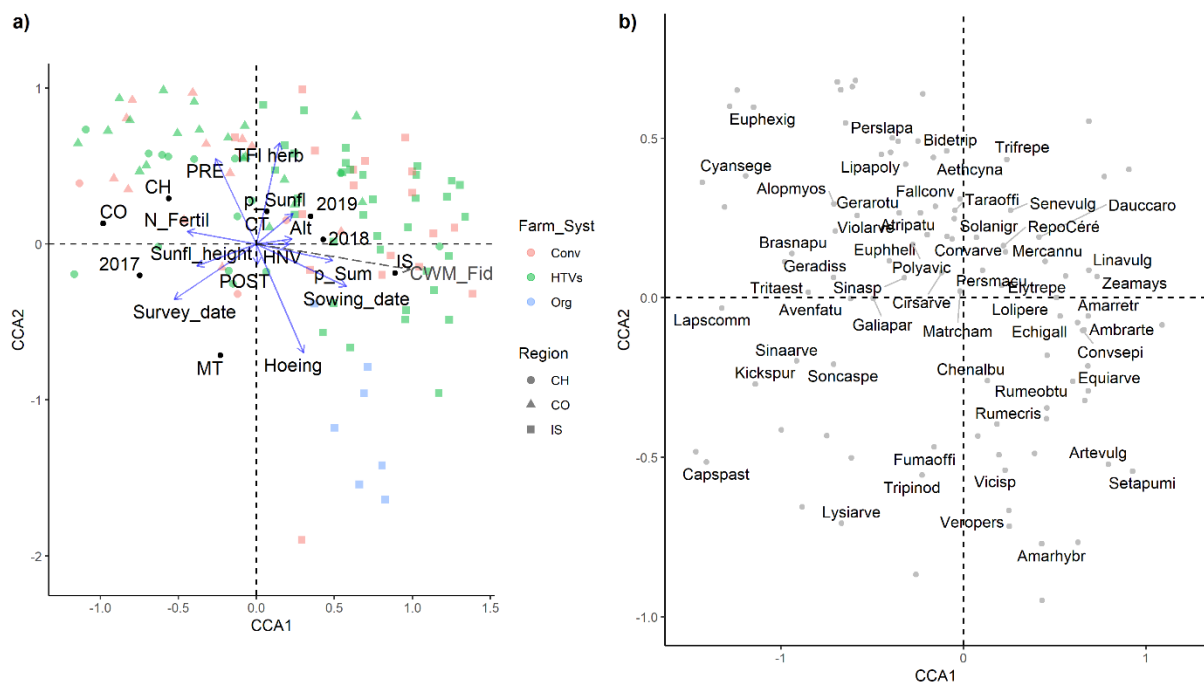


Figure 5. Percentage of explained variation for variables selected in the reduced model (left) and standardised effects of the fixed variables in the reduced model (right) for species richness (a, b), the exponential of Shannon’s diversity index (c,d) and the inverse Simpson index (e, f) of field margin plant communities. Variable definitions are given in Table 1.

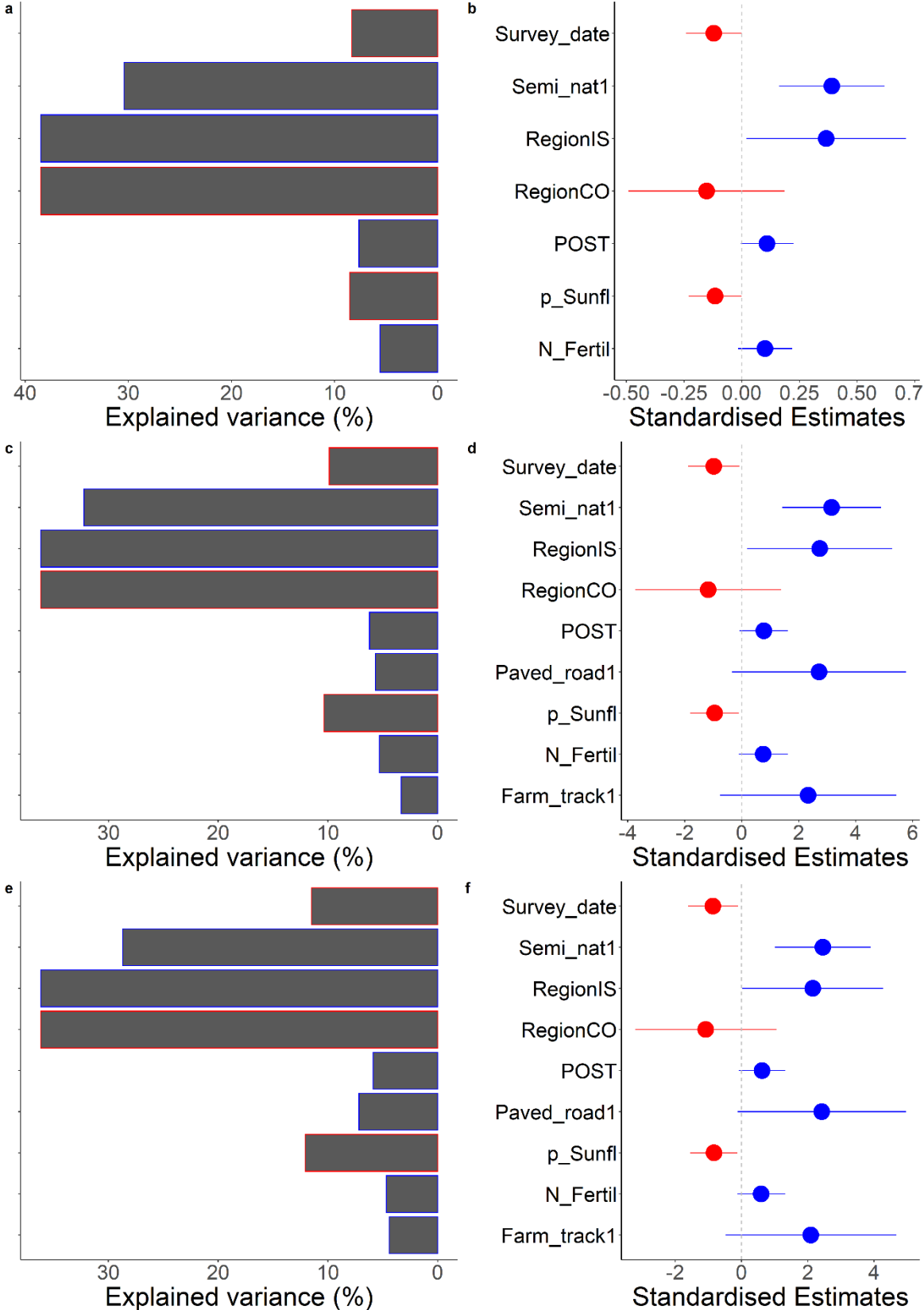


Figure 6. Ordination diagrams of the canonical correspondence analysis (CCA) model on field margins containing a) the sites and the explanatory variables, and b) the species (coded with the first four letters of the genus and species names). Colours indicate the three cropping systems (red: conventional, green: use of herbicide-resistant varieties, blue: organic) and shapes, the three regions (circles: Cher, triangles: Côte-d’Or, squares: Isère). The abbreviations used for the variables are defined in Table 1. The dashed vector represents the supplementary variable $CWM_{Fidelity}$ directed towards communities dominated by arable habitat specialists. The species names are only displayed for the species with the highest fit (other species are indicated by a grey circle).

