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# **Assessing vulnerability of functional diversity to species loss: a case study in Mediterranean agricultural systems**

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**Running headline: Vulnerability of Functional Diversity to species loss**

## **SUMMARY**

1. Increasing land use intensification is leading to biodiversity losses worldwide, which can reduce the functioning of ecosystems. However, it is increasingly clear that not all species are equally important for ecosystem processes: whereas the loss of a functionally unique species may reduce the capacity of the community to perform some functions, losing a functionally redundant species should have a much smaller impact.
2. Assessing the vulnerability of functional trait diversity (FD) to species extinctions can help to predict the impacts of land use intensification. This approach consists in ranking species according to their risk of extinction and then estimating the trajectory followed by FD as species are lost from local communities.
3. However, the most widely-used FD indices are not independent of species richness, being much more sensitive to the loss of species in species-poor than in species-rich sites. This may result in misleading interpretations, affecting our ability to rank communities according to the vulnerability of their FD to species loss, by confounding it with the initial level of species richness. Here we propose comparing the trajectory of FD under the most plausible order of species loss with that followed under random species losses as an effective way to remove the trivial effect of species richness in the assessments of vulnerability to species loss.

4. After decoupling vulnerability from species richness, we used it to analyse the effect of agricultural intensification on the vulnerability of arable plant communities in Mediterranean agricultural fields. Our results show that management strategies aiming to increase the functionality of these systems should focus on intermediately intensified fields, where small reductions in the level of intensification are likely to benefit arable plant diversity, increasing the number of species and FD and decreasing the vulnerability of FD to species losses.
5. Removing the effect of species richness is essential to attain unbiased estimations of the vulnerability of communities to species loss, especially when species-poor communities are considered. Combining vulnerability with information on taxonomic and functional diversity appears as a promising tool to inform decision-making processes, anticipating the effects of local extinctions.

**Keywords:** Agricultural intensification; Arable plants; Functional diversity; Mediterranean; Metacommunities; Species loss; Vulnerability.

## INTRODUCTION

One of the most common consequences of environmental changes is a reduction in the species richness of the affected communities (Bellard *et al.* 2012), which in turn can affect their functioning (Reich *et al.* 2012). This is especially true in agricultural landscapes, where demands for higher yield lead to the removal of unplanned associated biodiversity and reductions in delivered services (Tilman *et al.* 2002; Wood *et al.* 2015; Hevia *et al.* 2016). Agricultural intensification aims to increase yield through changes in management both at the local field and at the landscape level. Arable plants are one of the groups most notably affected by these practices (Guerrero *et al.* 2010; Storkey *et al.* 2012). Arable plants support services like biological pest control, as well as the presence of pollinators, birds and mammals (Marshall *et al.* 2003). A better understanding of the effects of agricultural intensification on the diversity of arable plant communities is crucial to design sustainable agricultural systems (Wood *et al.* 2015).

Functional trait diversity (FD) is considered a better proxy of ecosystem functioning than species richness (Tilman 1997; Loreau *et al.* 2001; Díaz *et al.* 2007). This is because not all species are functionally equivalent; species with similar traits play similar roles on ecosystem functioning, and hence can be considered as functionally redundant (Laliberté *et al.* 2010; Carmona *et al.* 2016). Consequently, the loss of a species that is functionally redundant with respect to a remaining one should have a lesser effect than the loss of a functionally unique species (Mouillot *et al.* 2013a). In this context, anticipating how the FD of communities will change with the extinction of taxonomic units is fundamental to understand their resistance to environmental changes.

However, estimations of functional redundancy at the community level ignore the fact that the order of species loss is not random (Zavaleta & Hulvey 2004). Rather, the likelihood of extinction of one species depends on features such as their resistance to stress or their rarity at the community or metacommunity level (Mouillot *et al.* 2013a; Rader *et al.* 2014; Sasaki *et al.* 2014). This key point should be considered when estimating the resistance of community functioning to species loss. Adopting a metacommunity framework allows estimating the most plausible order of species loss by considering nestedness (Rader *et al.* 2014; Sasaki *et al.* 2014). Species that are present in few sites (i.e. rare species -those at the right side in the nestedness matrix represented in Fig. 1a) are more prone to become extinct in the metacommunity after local extinctions, whereas more common species should be more persistent. This differential extinction of species may be reflected in local FD, as not all species are functionally equivalent. Consequently, estimating the most plausible order of species loss can help to describe expected changes in FD associated to local extinctions of species (Sasaki *et al.* 2014). Such changes are revealed by observing the trajectory of change in FD with species loss, simulating the extinction of individual species following the most plausible order of extinction. Following this reasoning, Sasaki *et al.* (2014) estimated vulnerability as the proportion of species that

need to be lost in a community to attain a substantial reduction in FD. Indeed, this vulnerability is determined by the relationship between species' risk of extinction and their level of functional redundancy.

However, this strategy can be misleading, because the rate of change of FD is generally not independent of the number of species (de Bello *et al.* 2016). For instance, the Rao and the FDis indices (Botta-Dukát 2005; Laliberté & Legendre 2010), probably the most widely used indices of FD, are much more sensitive to the loss of species in species-poor than in species-rich sites (Laliberté & Legendre 2010; de Bello *et al.* 2016). This means that changes in FD after the extinction of one species are likely to be larger in species-poor than in species-rich communities. Obviously, this can in turn affect our ability to rank communities according to the vulnerability of FD to species loss, by confounding it with the initial level of species richness. Comparing the trajectory of FD under the most plausible order of species loss with that followed under random species losses might be a good strategy to remove the trivial effect of species richness. For instance, let us consider a site in which the species that are more prone to extinction (according to the nestedness pattern) are also functionally unique with respect to the rest of the species in the local pool (Fig. 1a, Case A). In that case, we could expect a great reduction in FD after the extinction of only a few species, which makes the FD of such a site vulnerable to species losses (Fig. 1b, A). On the contrary, if the first species that are lost are functionally redundant with respect to other species (Fig. 1a, Case B), the FD of that site should be more resistant to species losses (Fig. 1b, B). Finally, if there is not a relationship between the redundancy of species and its extinction risk (Fig. 1a, Case C), the changes experienced by FD as species are lost should not differ from those under a random pattern of species extinction (Fig. 1b, C).

Agricultural intensification generally reduces species richness (e.g. Firbank *et al.* 2008; Geiger *et al.* 2010). However, the relationship between the resistance of communities' FD and agricultural intensification remains widely unknown. This information is potentially more relevant for the management of biological systems than the mere relationship of intensification with species richness or even with FD. Functional vulnerability allows forecasting the effects that changes in the local levels of land-use intensity (and associated changes in species richness) could have on the functioning of these communities, hence being a useful tool for decision-making, pointing to where these changes could have a greater impact.

This paper has two main objectives. First, to present a reliable framework to estimate the vulnerability of local FD to species extinctions that is independent of local species richness. Second, to characterize the patterns of vulnerability of arable plant communities across a land-use intensity gradient. To do this, we use data of arable plant communities encompassing a gradient of agricultural intensification, estimated at two relevant spatial scales (individual field and landscape levels), from a study area in central Spain. We calculate FD and its vulnerability to species loss for each field, and

then analyse its relationship with agricultural intensification at both spatial scales. Specifically, we want to find out 1) which are the patterns of variation of vulnerability across intensification gradients, 2) whether the relationship between vulnerability and intensification depends on the spatial scale at which intensification is observed, and 3) what is the shape of this relationship. Finally, we illustrate how to interpret the combined information provided by taxonomic diversity (TD), FD and vulnerability, to inform management decisions in agricultural systems.

## **MATERIALS AND METHODS**

### **Study area**

The study area occupies ca. 500 km<sup>2</sup> of flat to gently undulated dry cereal farmland in central Spain (40° 40' N, 3° 25' W; 600-800 m a.s.l.). Climate is Mediterranean, with mean annual temperature of 14.1 °C, average rainfall of ca. 400 mm, mostly concentrated in spring and autumn, and with very dry and hot summers. There are no important variations in climatic and soil characteristics or topographic features within the study area (Guerrero *et al.* 2014), where the dominant activity is rainfed cereal cropping (arable land covers ca. 86% of the area), and the landscape is a dynamic agricultural mosaic, formed by fields of varying sizes (between 0.5 and 30 ha). Further details can be found in Guerrero *et al.* (2014).

### **Vegetation sampling and agricultural management information**

We use the data presented in Guerrero *et al.* (2014), encompassing 78 agricultural fields sown with winter wheat within the studied area (see Appendix S1 in Supporting Information). In each of these fields, and according to their size, we placed between one and five sampling points at a distance of 10 m from the field margin (37 with one sampling point, 26 with two, 8 with three, 3 with four and 5 with five). In June 2007, we placed in each sampling point three 2x2 m sampling quadrats separated 5 m apart from each other parallel to the field margin. On each quadrat, we visually estimated the percent cover of each plant species; subsequently, we averaged the covers of the three quadrats of each sampling point, so that all analyses (species richness and FD) were performed at the sampling point level.

We measured six variables related to agricultural management, both related to the individual field (fertilizer input, sowing density, yield) and to the surrounding landscape (focal field size, mean arable field size and proportion of arable land cover; Appendix S2; Guerrero *et al.* 2010, 2012). We sent questionnaires to farmers to collect information on the three variables related to field management practices, and used digital maps created from remotely sensed images of the study area to estimate landscape structure variables within a radius of 500 m around each sampling point, using the average of each variable when there was more than one sampling point per field.

### **Extinction risk**

Following Sasaki *et al.* (2014) we tested whether the studied plant communities presented a nested

structure, so that the species from species-poor fields are a subset of those found in species-rich fields. If that was the case, the rank of the species in this nested structure (i.e. metacommunity) could be used as a proxy for the risk of extinction in local communities (Sasaki *et al.* 2014). In our study, sampling effort was not uniform across fields, with sampling points per field ranging from one to five. Uneven sampling effort can result in misleading estimations of the nestedness pattern, because fields with more sampling points are likely to have more species simply due to the higher sampling effort. Consequently, we performed a resampling procedure aimed to equalize the sampling effort on each field and hence get a correct *species x sites* matrix. We simulated 1,000 different resampling events, in which we randomly selected one sampling point per field, assembling a *species x sites* presence-absence matrix each time. For each resampling event, we estimated the nestedness of this matrix using the NODF measure (Almeida-Neto *et al.* 2008), which varies between 0 (no nestedness) and 100 (maximum nestedness). We evaluated the significance level of the NODF value attained on each resampling event using null model simulations (quasiswap option in the *oecosim* function of the ‘vegan’ package in R 3.2.0; Oksanen *et al.* 2015; R Core Team 2015). We performed 250 replicates of this null model for each of the resulting 1,000 matrices, and evaluated the proportion of them that displayed a significant level of nestedness ( $p < 0.05$ ).

For each resampling event, we determined the maximally nested *species x sites* matrix, where species and fields are ordered according to their incidence and species richness, respectively (Sasaki *et al.* 2014; Fig. 1a). We then ranked species according to its extinction risk, which increases as one species is present in a smaller number of communities (Fig. 1a). Finally, we used the average of the 1,000 ranks obtained in the resampling events to estimate the definitive extinction risk of species, which was later used to simulate the most plausible order of species loss in each community.

### Functional traits and vulnerability

We collected functional trait data for the species found in the vegetation survey. We selected four key traits –vegetative height (cm), Specific Leaf Area (SLA,  $\text{mm}^2 \text{mg}^{-1}$ ), seed mass (mg) and flowering onset (month)–, which are indicators of ecosystem functions, plant dispersal, establishment, persistence and response to disturbances (Cornelissen *et al.* 2003; Díaz *et al.* 2007). Functional trait information for 102 of the recorded species (representing on average 95% cover in the quadrats; Pakeman 2014) was extracted from the LEDA and e-FLORA-sys databases (Kleyer *et al.* 2008). Height, SLA and seed mass were log-transformed, and then, using Gower distances, we constructed a matrix of species functional dissimilarities considering the four traits together. The distances in the dissimilarity matrix range between 0 –when two species are functionally identical– and 1 –when two species have completely different traits. We then calculated the Rao index of diversity for each sampling point (Botta-Dukát 2005):

$$Rao = \sum_{i=1}^S \sum_{j=1}^S p_i p_j d_{ij} ,$$

where  $p_i$  and  $p_j$  are the relative abundances of species  $i$  and  $j$  in the considered sampling point, and  $d_{ij}$  is the functional dissimilarity between these species.  $FD_{Rao}$  is a combination of functional richness and functional divergence (Mouchet *et al.* 2010); it has the advantage that it can take species' abundances into account, and has been widely used in studies of functional diversity, including evaluations of vulnerability to species loss (Sasaki *et al.* 2014).

The value of  $FD_{Rao}$  for each sampling point represents the observed level of FD. Subsequently, for each sampling point, we simulated the loss of individual species (one species at a time) following the most plausible order of species loss, as explained in the previous section. After the loss of each species, we recalculated  $FD_{Rao}$ , and expressed it as a percentage of the original  $FD_{Rao}$  value. This allowed us to estimate the trajectory followed by the  $FD_{Rao}$  of each sampling point when species are locally extinct following the most plausible order (Fig. 1b). For each sampling site, we characterized the proportion of the original number of species that had to be lost to reduce the  $FD_{Rao}$  value in a substantial amount ( $P$ ), which we set at 25% (Fig. 1b) and 50%.

Then, we simulated the trajectory followed by  $FD_{Rao}$  under a random loss of species for each sampling point. This procedure was similar to the one described above, but now randomly selecting the species to be eliminated on each step. We repeated this procedure 1,000 times for each sampling point, hence attaining 1,000 values of  $P_R$ , which is the analogous of  $P$  under a random loss of species. We calculated the final  $P_R$  value of each sampling site as the average of these 1,000 values, with  $P_R$  representing the proportion of the original species that has to be lost randomly –with all species having the same probability to become extinct in each step– from a given site in order to reduce its original  $FD_{Rao}$  value by 25% (Fig. 1b) or 50%.

The values of  $P$  and  $P_R$  allowed us to calculate the richness-independent vulnerability of the functional diversity of each sampling point  $i$  (RIV) to species extinctions:

$$RIV_i = P_R - P$$

The logical behind the calculation of RIV is simple: if  $FD_{Rao}$  is reduced by local extinctions following the most plausible order in a faster fashion than what should be expected under random extinctions,  $P_R$  will be greater than  $P$ . Hence, RIV will be positive, indicating that local FD is more vulnerable to extinctions than what should be expected under random species losses (Fig. 1, case A). On the contrary, negative RIV values indicate that the FD of a site is less vulnerable than expected under random extinctions (Fig. 1, case B).

We finally averaged the vulnerability values obtained for the sampling points on a given field, obtaining one RIV value for each field. We removed fields with less than 3 species because of the potential uncertainty in the calculated value of RIV when the initial number of species is low, which left 77 fields for subsequent analyses.

## Data analysis

### *FD<sub>Rao</sub> and vulnerability indices vs. species richness*

To show the relationship between  $FD_{Rao}$  and species richness, we used the species pool from the



arable plant metacommunity to simulate 1,000 random communities for each possible value of species richness between 3 and 30 species. To remove the effect of species evenness from the results, the same abundance was assigned to all the species in a given community. The value of  $FD_{Rao}$  for each simulated community was calculated using the matrix of functional dissimilarities between species based on four traits.

For each community we then calculated its value of vulnerability following the method proposed in Sasaki *et al.* (2014) and the method presented above (RIV, see *Functional traits and vulnerability*). We compared the values of these two indices in relation to species richness by means of simulations. Sasaki *et al.* (2014) uses half-life –i.e. the proportion of the original species that has to be removed following the most plausible order of species loss to attain a 50% reduction of  $FD_{Rao}$  – as an estimator of vulnerability. By contrast, the method presented here compares half-life following the most plausible order of species loss with the average of the half-life values following a random order of species loss (RIV). To calculate this average, we simulated 1,000 random species losses for each simulated community in each level of species richness. In addition to the half-life values, we estimated the proportion of species that had to be lost to reduce the initial  $FD_{Rao}$  value by 25%, which was considered to be a relevant reduction in FD.

#### *Patterns of vulnerability across a land use intensity gradient*

Following Guerrero *et al.* (2014), we applied Principal Component Analysis (PCA) to the agricultural management variables (Appendix S2), obtaining two orthogonal axes related with intensification at the individual field and at the landscape levels, respectively. Then, we regressed vulnerability (RIV), functional diversity ( $FD_{Rao}$ ) and taxonomic diversity (TD; Simpson index of diversity; see de Bello *et al.* 2016) values of each field against the two indicators of agricultural intensification (two principal axes in the PCA). Additionally, we studied the relationship between functional redundancy and RIV. To do so, functional redundancy was estimated by subtracting the value of  $FD_{Rao}$  to the value of TD in each community (de Bello *et al.* 2007; Pillar *et al.* 2013).

Because FD often correlates in a non-linear fashion with intensification (Flynn *et al.* 2009; Laliberté *et al.* 2010; Guerrero *et al.* 2014), we used the R package mgcv to fit Generalized Additive Models (GAM; Wood 2006), thus accounting for non-linear relationships between agricultural intensification, RIV,  $FD_{Rao}$ , and TD. To avoid overfitting, the basis dimension parameter (k) was set to a maximum of 5, defining an upper limit of 4 degrees of freedom associated with the smooth estimation. We initially included field-level and landscape-level intensification as explanatory variables in the analyses, and then removed the non-significant terms. We used the same strategy to study the relationship between RIV and redundancy. We used Mantel tests to check for potential spatial autocorrelation in the residuals of all models. All analyses were performed using the R software (version 3.2.0; R Core Team 2015).

## RESULTS

A great majority (75.6%) of the metacommunities had NODF values significantly higher than random expectations ( $\text{NODF} = 29.52 \pm 0.06$ ), indicating that the studied communities had a nested structure, with the species-poor communities being a subset of the species-rich ones (Fig. 1a).

Our simulations confirmed that functional diversity ( $\text{FD}_{\text{Rao}}$ ) is not independent of species richness, but rather follows an asymptotic function, with a much stronger relationship for low values of species richness than for high ones (Fig. 2a). Consequently, losses (or gains) of species are likely to have a greater impact on the  $\text{FD}_{\text{Rao}}$  values of species-poor communities than on those of species-rich communities. This was corroborated by the relationships between the vulnerability values calculated following the half-life method (Sasaki *et al.* 2014) and richness independent vulnerability (RIV). For both thresholds of reduction in  $\text{FD}_{\text{Rao}}$  (25% and 50%), the vulnerability values calculated using half-life were not independent of species richness, but rather mirrored the relationship between  $\text{FD}_{\text{Rao}}$  and species richness (Fig. 2b). By contrast, RIV was independent of species richness for both thresholds (Fig. 2c).

The GAM model including both predictors revealed that agricultural intensification at the landscape level did not have a significant effect on any of the analysed variables. Consequently, this variable was removed from the final models, and all subsequent results refer to models including only intensification at the individual field level. Mantel tests did not detect significant spatial autocorrelation in any of the models ( $r = -0.031$ ,  $p = 0.778$  for TD;  $r = -0.036$ ,  $p = 0.731$  for FD; and  $r = 0.022$ ,  $p = 0.240$  for RIV). TD decreased linearly with intensification (1,00 estimated degree of freedom;  $F = 27.73$ ;  $p < 0.001$ ; Fig. 3a).  $\text{FD}_{\text{Rao}}$  showed a marked non-linear pattern, with high values in the least intensified fields that decreased rapidly at intermediate levels of intensification and stabilized again at the most intensified end of the gradient (3.03 e.d.f.;  $F = 8.92$ ;  $p < 0.001$ ; Fig. 3b). Intensification at the field level explained 21.10% and 28.49% of the total variance in RIV for the 50% and 25% thresholds, respectively. The relationship between RIV and intensification at the individual field level was also non-linear (2.09 e.d.f.,  $F = 8.35$ ,  $p < 0.001$  for the 50% threshold; 2.13 e.d.f.,  $F = 10.46$ ,  $p < 0.001$  for the 25% threshold). Because the models for both thresholds presented an almost identical shape, we will only refer onwards to the 25% threshold one. Importantly, RIV in the least intensified fields was much lower than randomly expected (Fig. 3c). In these conditions (low values of field-level intensification), RIV increased with intensification, but this relationship disappeared under higher levels of intensification (Fig. 3c).

The relationship between RIV and redundancy was negative and slightly non-linear (1.53 e.d.f.;  $F = 14.89$ ;  $p < 0.001$ ; Appendix S3). Importantly, the strength of the relationship between the two variables was relatively low (redundancy explained 26.43% of the total variance in RIV), confirming that the two indices represent different aspects of communities.

## DISCUSSION

In this paper, we present a method to estimate the vulnerability of the FD of biological communities to species loss that is independent of species richness, as well as an evaluation of the effect of agricultural intensification on such vulnerability. We showed that a previous estimator of vulnerability (Sasaki *et al.* 2014) have the same intrinsic relationship with species richness than  $FD_{Rao}$  and, consequently, it is likely to estimate smaller vulnerabilities for species-rich communities. The new vulnerability index (RIV) that we propose in this paper has demonstrated to be independent of species richness and seems a good tool for analysing the effect of agricultural intensification on arable plant communities. Our results show that intensification at individual field level played an important role as a determinant of vulnerability in arable fields. Specifically, the potential effect of species extinctions on  $FD_{Rao}$  was more important in the most intensified fields, while the FD of the least intensified fields was more resistant to the loss of species. Importantly, this response was markedly non-linear, with the greatest effects being under low intensification conditions, where RIV rapidly increased with intensification. This in turn implies that the lower FD vulnerability to species losses of the least intensified fields is rapidly reduced, even when intensification increases slightly. In this section we discuss these results in terms of the functional redundancy of the pool of species of arable plant communities, and propose a framework including TD,  $FD_{Rao}$ , and RIV to inform decision-making processes.

### Redundancy and vulnerability

Functional redundancy within communities is considered as an insurance against the loss of ecosystem functions after the extinction of species (Walker 1995; Moretti, Duelli & Obrist 2006; Pillar *et al.* 2013). The most common approaches to estimate redundancy are based either on the use of functional groups (Walker, Kinzig & Langridge 1999; Laliberté *et al.* 2010), or on the analysis of the covariation between species richness and a dissimilarity-based index of functional diversity (Sasaki *et al.* 2009; Guerrero *et al.* 2014). However, both alternatives have a series of drawbacks (reviewed in Carmona *et al.* 2016). Recently, it has been proposed to define redundancy as the fraction of species diversity not expressed as functional diversity (de Bello *et al.* 2007; Pillar *et al.* 2013).

In any case, indicators of redundancy are not necessarily good predictors of the resistance of ecological function to species loss, because they do not consider the different risk of species extinction. As a consequence, two communities with the same species (and the same value of redundancy) can show very different values of vulnerability, depending on which species are more prone to become extinct (Mouillot *et al.* 2013a). Our results confirm this point, showing that, despite the logically expected negative relationship between RIV and redundancy (i.e. the  $FD_{Rao}$  of communities with more redundant species is relatively resistant to species loss, and vice versa), both indices provide different information. In fact, redundancy decreased linearly along the intensification

gradient (Appendix S4), contrasting with the non-linear response of RIV. Consequently, when the extinction risk of species can be estimated, we recommend to use RIV rather than redundancy as a more reliable indicator of the resistance of communities to species loss.

### **A richness-independent measure of vulnerability**

The proportion of the original number of species that have to be removed following the most plausible order of species loss to attain a substantial reduction of  $FD_{Rao}$  is a promising approach to estimate vulnerability. Compared with redundancy indices, the consideration of the risk of extinction of species is conceptually a major step forward to estimate the vulnerability of communities and its changes across environmental or land-use gradients (Sasaki *et al.* 2014). However, the results of our simulations show that the  $FD_{Rao}$  half-life approach to measure vulnerability is not independent of the original number of species. This lack of independence is inherited from the relationship between  $FD_{Rao}$  and species richness (de Bello *et al.* 2016).  $FD_{Rao}$  is not linearly related with species richness, but rather follows an saturating curve (Laliberté & Legendre 2010; de Bello *et al.* 2016). As a result of this relationship, the  $FD_{Rao}$  value of a community with low species richness can be much more sensitive to the addition or subtraction of one species than that of a community with high species richness (Fig. 2a). Consequently, the straightforward use of  $FD_{Rao}$  half-life as a proxy for vulnerability may lead to unclear ecological conclusions, by considering species-rich communities as less vulnerable than species-poor ones (Fig. 2b). In this paper we show a new alternative, comparing the proportion of species that has to be lost to attain a given reduction in  $FD_{Rao}$  with the proportion that has to be lost following a random order of extinction. Given that RIV is independent of species richness (Fig. 2c), it could be considered as a more reliable indicator of the vulnerability of each community to the loss of species than  $FD_{Rao}$  half-life (Sasaki *et al.* 2014), especially when there are communities with a low number of species (Fig. 2).

### **Vulnerability of arable plant communities across intensification gradients**

The results for arable plant communities revealed important changes in the levels of vulnerability associated with agricultural intensification, which implies that species differed in terms of their functional redundancy. The less intensified fields displayed negative RIV values. These values were originated by substantial deviations of the trajectory followed by  $FD_{Rao}$  after a plausible order of species extinction from that under a random order of species loss, resulting in a greater than expected resistance of  $FD_{Rao}$  (Appendix S5). This implies that the species with the highest risk of extinction in these fields are functionally redundant, resulting in small decreases of  $FD_{Rao}$  after the loss of the first few species (Fig. 1, Case B). On the other hand, in fields experiencing higher levels of intensification, the trajectory of  $FD_{Rao}$  after a plausible order of species loss did generally not differ from the trajectory after random species loss. This indicates that the most extinction-prone species are neither unique nor redundant with respect to the rest of the species in the local pools of those fields (Fig. 3).

Our results suggest that field-level agricultural intensification does not only reduce TD and  $FD_{Rao}$  in arable plant communities (Guerrero *et al.* 2014), but also eliminates functionally redundant species,

thus increasing the vulnerability of the community to species loss. Most importantly, patterns along the intensification gradient for  $FD_{Rao}$ —greater decreases at intermediate levels of intensification— and TD—linear decrease— contrasted with the increase in RIV at the first stages of intensification, showing that TD,  $FD_{Rao}$ , and RIV yield complementary information. Combining these three sources of information appears as a very powerful tool to evaluate different management alternatives (Mouillot *et al.* 2013b). In the presented case, it seems that management strategies aiming to increase the functionality of these systems should focus on fields with intermediate levels of intensification. Even small reductions of the level of intensification in these fields are likely benefit arable plant diversity, increasing TD and  $FD_{Rao}$ , and decreasing vulnerability. On the contrary, the most intensified fields would require great reductions in the level of intensification to increase  $FD_{Rao}$  and decrease its vulnerability, appearing as a much less effective management alternative. The inclusion of RIV is also a key aspect in the evaluation of the effects of increased intensification. For example, one approach considering only  $FD_{Rao}$  would suggest that increasing intensification in the least intensified fields is not especially detrimental (Fig. 3b). However, the extra information provided by RIV shows that, even if there are not reductions in  $FD_{Rao}$ , the vulnerability of these fields increases rapidly with intensification (Fig. 3c), so that any action increasing intensification levels should be observed with caution.

One important point to raise is the use we have made of trait information retrieved from public databases. Due to the effect of intraspecific variability, local measurements are generally preferable, but achieving such level of detail can be much more resource-consuming. However, the relative importance of considering local trait values, critical when considering effects at the plot scale (Carmona *et al.* 2015), decreases at the regional scale (Cordlandwehr *et al.* 2013), which is the case of the present study. It has been shown that species rankings considering traits from databases and local measurements are similar (Kazakou *et al.* 2014), thus suggesting that the conclusions presented in our case study should be robust to this problem.

## CONCLUSIONS

Current rapid environmental changes are leading to major biodiversity loss and alterations in the composition and relative abundance of species within communities. Such changes are likely to have a major impact on ecosystems, by reducing their resilience to environmental change and their capacity to provide multiple ecosystem services for humans. In order to face this problem, the development of comprehensive tools and programs to monitor the biodiversity, vulnerability and services provided by different ecosystems appears as an essential need (de Bello *et al.* 2010). The functional aspect of diversity encompasses several concepts, which implies that its study requires the adoption of a combination of approaches and indices (Mouillot *et al.* 2013b; Mason *et al.* 2013; Carmona *et al.* 2016). The RIV index presented in this paper has potential to become a useful tool for the monitoring

of ecological communities.

Most importantly, we have shown that RIV is independent from species richness and also from the observed levels of  $FD_{Rao}$  in each community. Consequently, combining RIV and  $FD_{Rao}$  is a powerful alternative to inform decision-making processes and anticipate the effects of local extinctions (Mouillot *et al.* 2013b). The adoption of such a framework would permit to focus conservation actions on sites with high values of  $FD_{Rao}$  and vulnerability, at the expense of others with low  $FD_{Rao}$  and high vulnerability or high  $FD_{Rao}$  and low vulnerability. Moreover, as suggested by Sasaki *et al.* (2014), the approach presented here can also be applied using taxonomic or phylogenetic rather than (or in combination with) functional diversity, particularly in the cases with no trait information available (Flynn *et al.* 2011). This results into a highly flexible framework for analysing and predicting the effects of environmental changes and associated species loss on diversity and ecosystem functioning.

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## DATA ACCESIBILITY

Trait data is already available in Guerrero *et al.* (2014). Data on species identities and relative abundances of species on each sampling point of each field have been deposited in the Dryad Digital Repository doi:10.5061/dryad.2td06 (Carmona *et al.* 2016)

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## **SUPPORTING INFORMATION**

Additional supporting information may be found in the online version of this article.

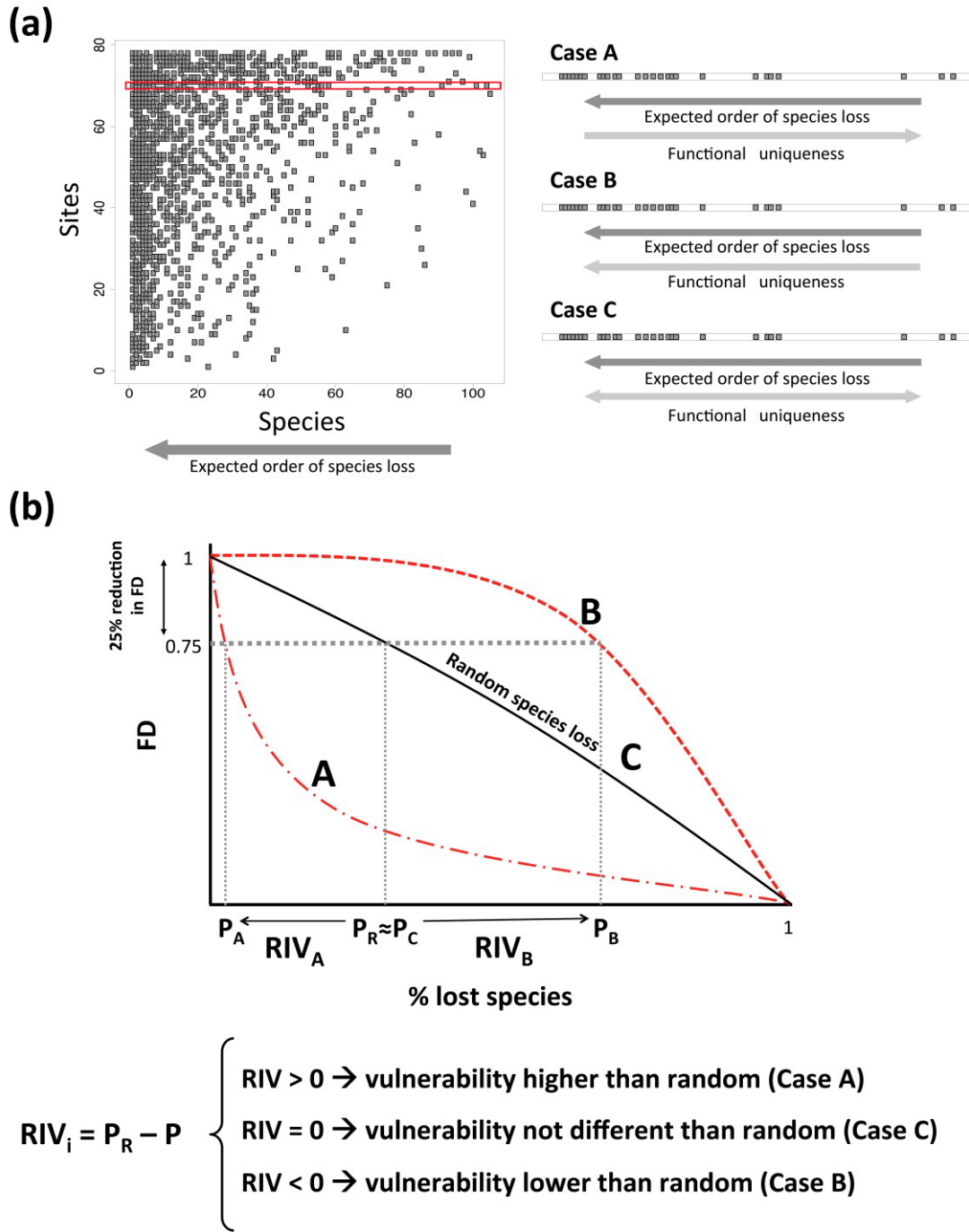
**Appendix S1.** Location of the sample fields within the study area

**Appendix S2.** Principal Component Analysis of agricultural management

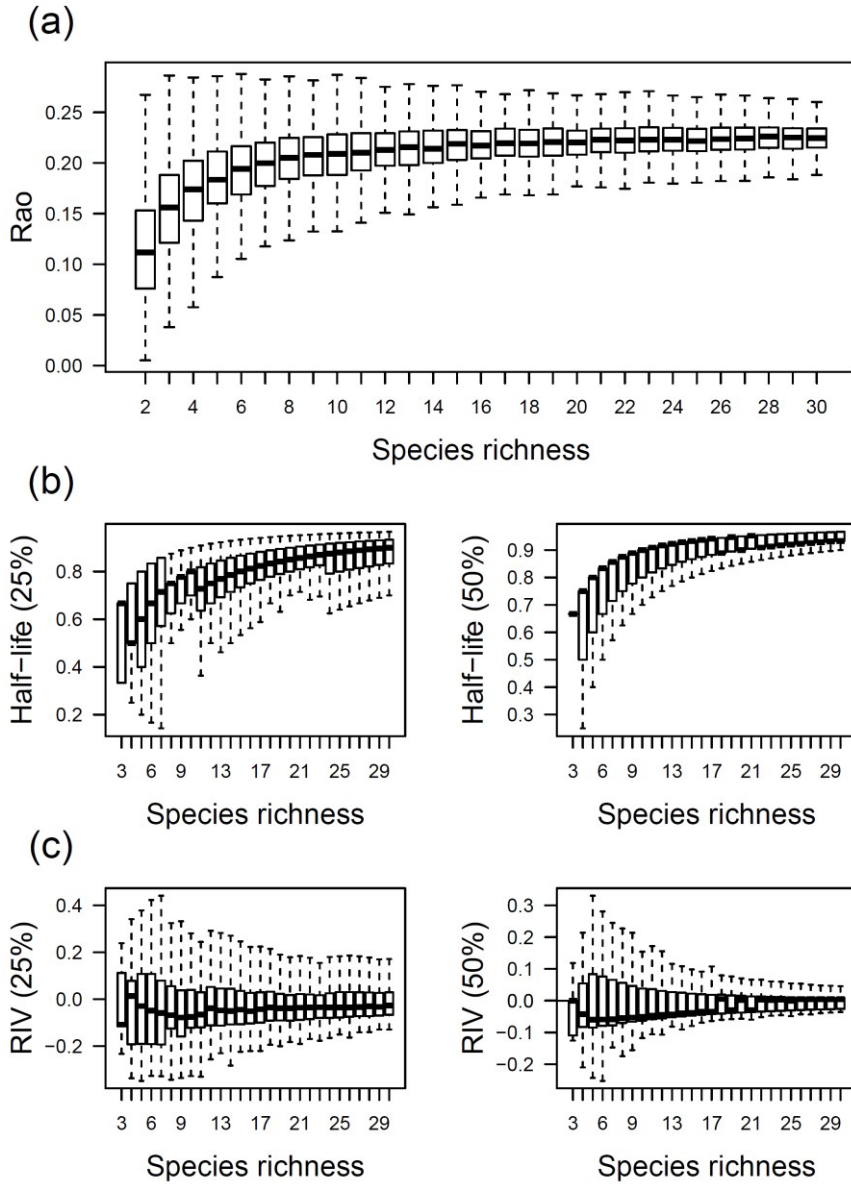
**Appendix S3.** Relationship between functional redundancy and richness independent vulnerability.

**Appendix S4.** Relationship between functional redundancy and agricultural intensification

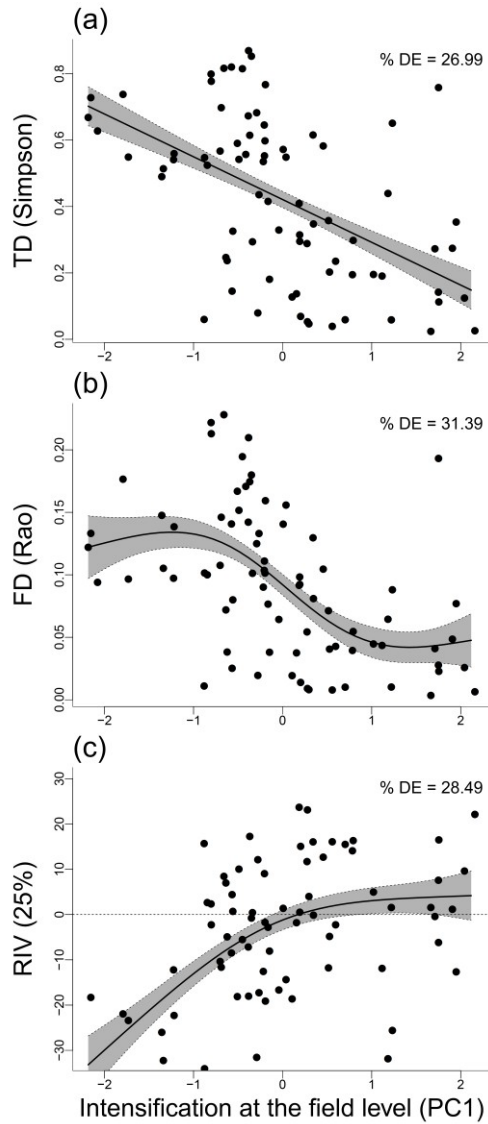
**Appendix S5.** Examples of trajectories of FD.



**Fig. 1.** Schematic representation of the framework for estimating the vulnerability of functional diversity (FD) to species loss. (a) The nestedness matrix can be used to estimate the most plausible order of species loss, with the species that are present in fewer sites having the greater risk of extinction and vice versa. (b) The degree of functional uniqueness with respect to the rest of species in the local pool interacts with the order of extinction to determine the vulnerability of communities. Vulnerability can be decoupled from species richness by comparing the rate of FD decrease under the most plausible order of species loss ( $P$ ) with the rate of decrease under random extinctions ( $P_R$ ), thus obtaining the richness-independent vulnerability index (RIV).

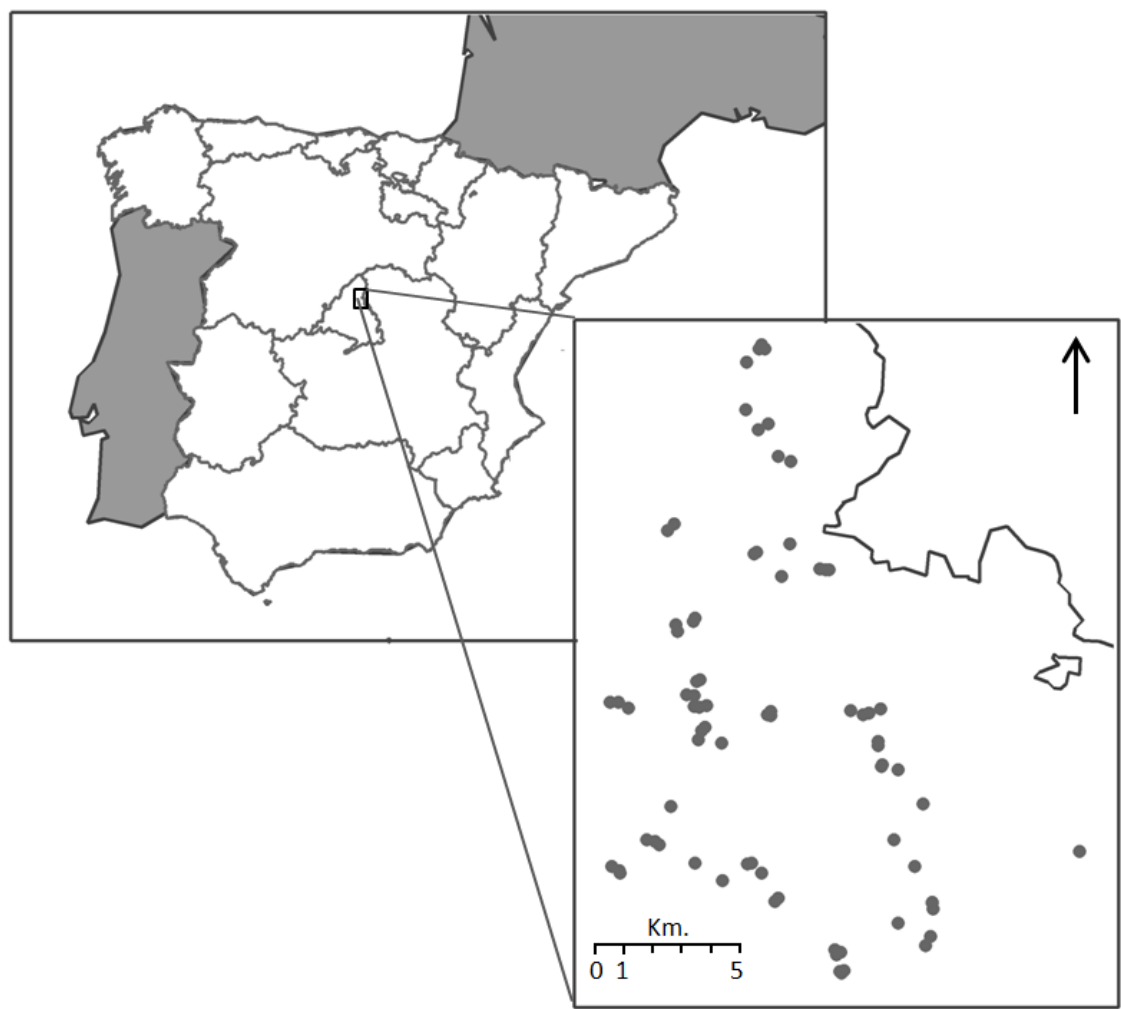


**Fig. 2.** Functional diversity and vulnerability indices in relation with species richness. Simulations of 1,000 random communities for each possible value of species richness between 3 and 30 species (see main text for details), showing that the Rao index of functional diversity (a) is not independent of species richness but rather displays a non-linear relationship. This relationship is inherited by the half-life method to estimate the vulnerability of functional diversity (b). However, comparing half-life values with those expected under a random order of species loss (richness-independent vulnerability, RIV) removes this bias (c).



**Fig. 3.** GAM models representing the relationship between agricultural intensification at the individual field level (PC1) and taxonomic diversity (TD) (a), functional diversity (FD) (b) and richness-independent vulnerability (RIV) (c). The proportion of deviance explained by the corresponding GAM model is indicated in each panel. Shaded areas indicate the 95% confidence interval of the regressions.

**Appendix S1.** Location of the sample fields within the study area.



## Appendix S2. Principal Component Analysis of agricultural management

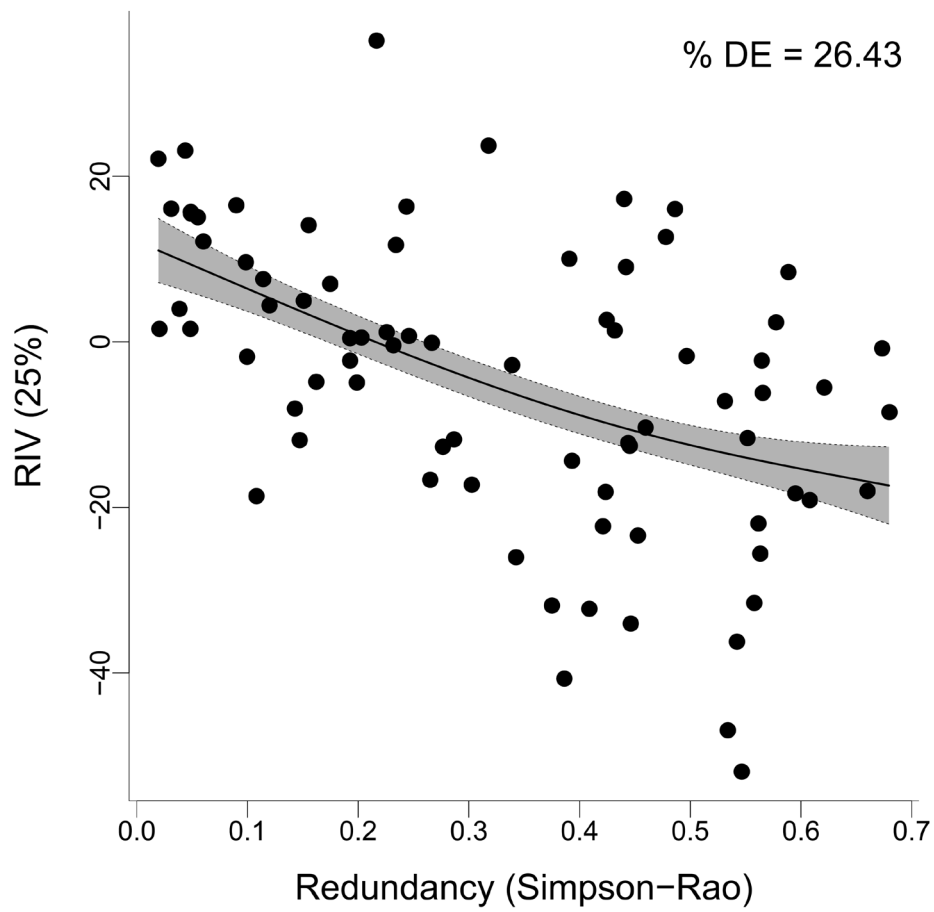
We applied Principal Component Analysis (PCA) to the agricultural management variables, explaining 63% of the variance in these variables. The first axis (PC1) was contributed by Yield, Fertilizer and Sowing Density, and can be understood as an indicator of intensification at the individual field level. The second axis (PC2) was contributed by Focal field size, Mean arable field size and Proportion of arable land, thus being an indicator of agricultural intensification at the landscape level (Table A1).

**Table A1.** Description and summary statistics of field management and landscape level variables used to characterize sampled cereal fields ( $n = 78$ ) and Principal Component Analysis loadings in factors summarizing field-level (PC1) and landscape-level (PC2) management characteristics of sampled fields.

Variable	Description	Mean $\pm$ SD	PC1	PC2
<i>Field management</i>				
Fertilizer	Total kg/ha nitrogen applied on focal field	59.47 $\pm$ 33.46	0.797	0.125
Sowing density	Density (kg/ha) of seed sown	204.17 $\pm$ 70.23	0.682	0.010
Yield	Cereal grain (ton/ha) obtained in focal field	3.02 $\pm$ 1.22	0.832	-0.127
<i>Landscape characteristics</i>				
Field size	Focal field size (ha)	4.47 $\pm$ 5.24	0.127	0.870
Mean field size	Mean size (ha) of fields with arable crops within a circle radius 500 m centered in the sampling point	3.54 $\pm$ 4.21	0.050	0.857
Arable land cover	Percentage of cultivated land within a circle radius 500 m centered in the sampling point	62.08 $\pm$ 26.27	-0.383	0.519
Proportion of variance explained			0.326	0.299
Cumulative variance explained			0.326	0.625

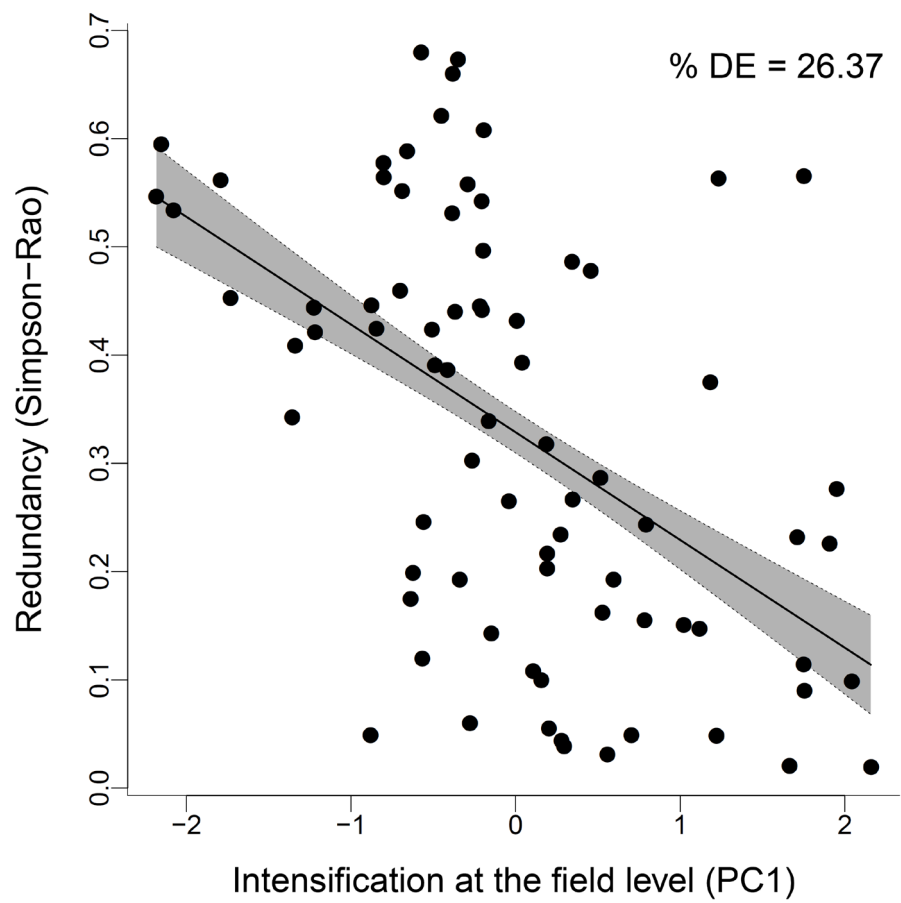
All variables adjust to a normal distribution (Kolmogorof-Smirnov test,  $P < 0.05$ ) except Sowing density and Mean field size, which were  $\ln(x+1)$  transformed.

**Appendix S3.** GAM model representing the relationship between functional redundancy and richness independent vulnerability (RIV). %DE indicates the proportion of deviance explained by the GAM model. Shaded areas indicate the 95% confidence interval of the regression.





**Appendix S4.** GAM model representing the relationship between functional redundancy and agricultural intensification. %DE indicates the proportion of deviance explained by the GAM model. Shaded areas indicate the 95% confidence interval of the regression.



**Appendix S5.** Examples of trajectories of FD after the most plausible order of species loss (red lines) and one random order of species loss (black lines). Communities are ordered in an increasing order of richness independent vulnerability (RIV), which was estimated for each community by the average difference of 1,000 simulated random extinction trajectories with the trajectory under the most plausible order of species loss (see main text for further details).

