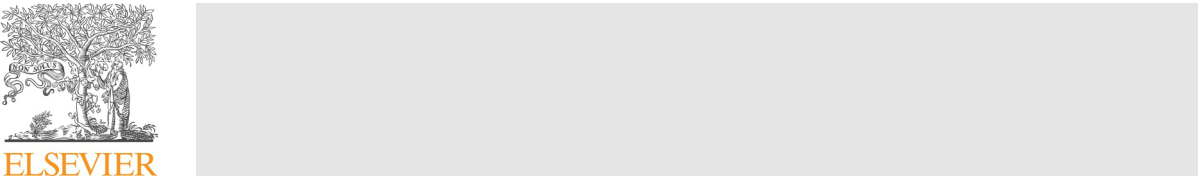
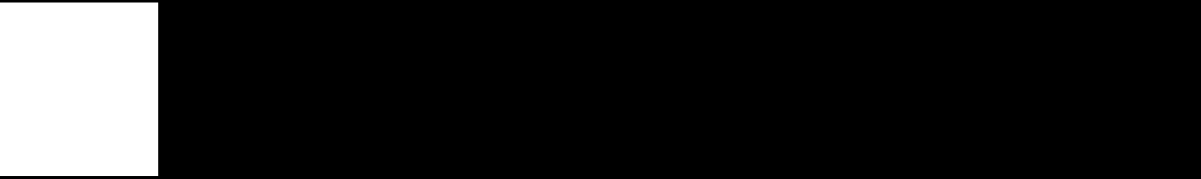
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Floral trait functional diversity is related to soil characteristics and positively influences pollination function in semi-natural grasslands

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ABSTRACT

Semi-natural grasslands are threatened habitats providing many ecosystem services, such as climate regulation or water erosion control. Factors affecting the vegetative trait diversity of their plant communities are well studied, but those affecting their floral diversity are not. Local factors, such as land-use intensification and soil characteristics, can greatly influence floral trait diversity in semi-natural grasslands, which in turn can influence pollination function. We selected 16 semi-natural grasslands in France along a local land-use intensification gradient. Floral functional diversity indices were calculated from measurements of five floral traits known to influence interactions with pollinators: flower area, flowering height, floral reflectance, nectar tube depth and nectar sugar production per floral unit. Using linear mixed models, we found a negative influence of phosphorus soil content, and a positive influence of a composite soil variable including soil texture, organic carbon and total nitrogen soil content, on floral functional diversity. However, local land-use intensification did not influence floral functional diversity. Moreover, our results showed a positive relation between floral functional diversity and plant-pollinator interaction frequency as a proxy of pollination function. More specifically, we found a positive influence of the nectar resource diversity (i.e. the variability in production of nectar sugar by flowers) on plant-pollinator interaction frequency. These results, based on a correlative approach and functional diversity indices as measure of functional complementarity, suggest that the diversity of floral traits may promote niche partitioning of pollinators and pollination function approximated by plant-pollinator interaction frequency, and highlight the main role of nectar in this process.

**1. Introduction**

Semi-natural grasslands (hereafter, “grasslands”) are major ecosys-tems and play an essential role in delivering ecosystem services. In Europe, half of the flora depends on grassland habitats (Veen et al., [2009](#page12)). Grasslands are threatened habitats, notably due to intensifica-tion of agricultural practices (Huyghe et al., 2014). They are covered by herbaceous plants, whose community structure and composition are driven in part by pollinators (Lundgren et al., 2016). In turn, in the context of global pollinator decline (Potts et al., 2010), grasslands may be suitable habitats for pollinators and may supply pollen and nectar resources for them (Woodcock et al., 2014a). Landscape context has been highlighted as a main driver of pollinator abundance and diversity



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in natural habitats (e.g. Steffan-Dewenter et al., 2002; Gámez-Virués et al., 2015), because pollinator foraging distances range from a few hundred meters to a few kilometers (Greenleaf et al., 2007). However, local land-use intensification at within-field grassland scale also need to be considered (e.g. Weiner et al., 2014). Local land-use intensification can even more influence pollinator community composition than landscape context (e.g. [Kormann et al., 2015](#page11)).

Indeed, grassland species diversity and total flower cover are known to positively influence pollinator abundance and diversity (e.g. [Ebeling](#page10) et al., 2008; Hudewenz et al., 2012; Woodcock et al., 2014a). Grassland plant species diversity and total flower cover levels may be determined by local environmental factors, especially land-use intensification which expresses for instance an increase in mowing frequency,

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fertilization and stocking rate (hereafter, “intensification”) ([Gaujour](#page11) et al., 2012), and soil characteristics (Ceulemans et al., 2013; [Grace](#page11) et al., 2016; Janssens et al., 1998). Intensification and soil character-istics can be related, even though soil characteristics may reflect past land use rather than current intensification (e.g. soil fertility via phos-phorus content: Ceulemans et al., 2014). Furthermore, local environ-mental factors like intensification and soil characteristics can also in-fluence floral functional diversity due to their influences on plant community structure, on phenotypic expression of floral traits of the same species, or a combination of these two mechanisms.

Floral traits lie at the core of plant-pollinator interactions. They are considered “matching traits” (Armbruster, 2017), which mediate rela-tions between two trophic levels (Le Provost et al., 2017) and are a main component of plant-pollinator interaction patterns ([Vázquez](#page12) et al., 2009a). Along with phenology (Vázquez et al., 2009b), they in-clude cues or signals (e.g. flower colour, which can match pollinator visual systems; van der Kooi et al., 2019), exploitation barriers (e.g. nectar tube depth, which can match lengths of pollinator mouthparts; Klumpers et al., 2019), and rewards (e.g. sugar production, which can match pollinator metabolic needs; Vaudo et al., 2015). A holistic view of floral traits is needed to understand plant-pollinator interactions. Ideally, floral traits should be measured quantitatively to be more ac-curate and consider pollinator matching and perceptions (Junker and Parachnowitsch, 2015). Quantitative measurements of this combina-tion of traits are found almost only for experimental sites or sites that do not represent natural habitats (e.g. Fornoff et al., 2017; Junker et al., [2013](#page11)), notably due to the costly and time-consuming effort needed to measure them.

Few studies in the literature identify relations between local en-vironmental variables and floral trait functional diversity indices. However, increasing intensification has been shown to decrease flower colour functional diversity (based on Euclidean distance in a Maxwell triangle; Binkenstein et al., 2013) and mean flowering height in grassland plant communities (Binkenstein et al., 2013; Garnier et al., [2007](#page11)). Furthermore, both intensification and increase in soil fertility could decrease floral functional diversity because of the positive cor-relation between functional diversity indices and plant species richness ([Fornoff et al., 2017](#page10)), but these influences have not been tested to date.

By influencing the value of floral trait functional diversity indices in plant communities, intensification and soil characteristics can have important cascading effects on the pollination function (Lavorel et al., [2013](#page11)), often approximated by the number or frequency of plant-polli-nator interactions (Ballantyne et al., 2017; hereafter “interaction fre-quency”). However, there is no consensus on how functional diversity of floral traits influences interaction frequency. Indeed, according to positive relations between biodiversity and ecosystem functioning, in-crease in floral functional diversity should lead to an increase in niche partitioning between pollinators and hence an increase in interaction frequencies (Schleuning et al., 2015). It relies on the assumption that for a given number of pollinators species available in a landscape, the more plant species are complementary on the basis of the value of their floral traits in a given grassland community, the more pollinator species find and interact with a flower with which they match.

In contrast, an increase in floral functional diversity was found to decrease interaction frequencies (Fornoff et al., 2017; [Uyttenbroeck](#page11) et al., 2017), partly due to an increase in the complexity of commu-nication between plants and pollinators, making it more difficult for pollinators to detect flowers (cf. serial vs. parallel processing; [Chittka](#page10) and Raine, 2006). Furthermore, Fornoff et al. (2017) did not distinguish floral trait functional diversity indices from flowering plant species richness, which may limit understanding of the true importance of floral functional diversity in plant-pollinator interactions. This limita-tion can be overcome by using null models (Swenson, 2014). Finally, a decrease in total flower cover and mean values of floral traits related to visual attractiveness (e.g. flower area and height) and reward avail-ability (e.g. sugar production by nectar) should theoretically lead to a

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decrease in interaction frequencies (Fornoff et al., 2017; Hegland and Totland, 2005). However, relatively few studies examine these rela-tions.

We studied 16 French grasslands to examine cascading effects of intensification and soil characteristics on relations between floral traits and the pollination function, approximated by interaction frequency. More precisely, we aimed to study (i) influences of intensification and soil characteristics on floral functional diversity indices and on total flower cover, (ii) influences of floral functional diversity indices and total flower cover on pollination function, approximated by interaction frequency, in grasslands. We hypothesised that intensification and soil fertility (i.e. total nitrogen and phosphorus soil content) decrease functional diversity of floral traits but also decrease visual attractive-ness and reward availability in grasslands due to a decrease in floral trait mean value and total flower cover. In turn, we hypothesised that decline in functional diversity of floral traits, visual attractiveness, and reward availability decrease the interaction frequency. In our study, floral functional diversity is only considered as a result of change in plant community structure, and phenotypic expression of floral traits of the same species is not taken into consideration.

**2. Materials and methods**

*2.1. Study sites*

We selected 16 grasslands in a 12 km² circle centred on Sarrebourg (48°73 N, 7°05E, 250 m a.s.l.) in the department of Moselle, France (Table A.1). Moselle’s climate is semi-continental, with a relatively wide temperature range between winter and summer (Δ14.6 °C be-tween summer and winter on the period 1981–2010, Météo-France). From April-July (i.e., the growing season) 2017, mean daily tempera-ture was 15.3 ± 5.7 °C, and precipitation was 290.9 mm (+0.7 °C and -15.9 mm compared to the period 1981–2010, Météo-France). To focus on local within-field intensification effects, we aimed to standardise the influences of landscape context by selecting grasslands on the basis of their landscape features. Shackelford et al. (2013) reported that above a threshold of 30 % of semi-natural habitats in the landscape (semi-nat-ural grasslands, orchards, hedgerows, groves, and forest edges; here-after “SNH”), the influence of the % SNH on pollinator abundance and diversity is weakening. Using a Geographic Information System (QGIS) and land-cover data from BD TOPO® (IGN) 2014 and RPG (IGN) 2014, we thus selected grasslands with more than 30 % semi-natural habitats of land cover in a buffer of 1000 m around each grassland centroids (range = 32–70 %). Furthermore, we established a threshold of less than 30 % of crop area (range = 0.7–25.0 %) to minimise influences of crops on plant and pollinator communities, and a minimum buffer of 200 m between grassland and crop edges to avoid aerial dispersal of pesticides ([Jong et al., 1991](#page11)).

Although our study focused on local environmental factors, we characterised the landscape context of the study sites by computing three metrics at a radius of 1000 m around grasslands in order to ensure that the effects we observed are not driven by the landscape context: total crop cover (Heard et al., 2007); total SNH cover (Steffan-Dewenter et al., 2002) and overall habitat diversity (Krauss et al., 2004). To calculate overall habitat diversity, we used a Shannon-Wienner index for each of the 16 grasslands (Krauss et al., 2004). The different habitats taken into account were semi-natural grasslands, orchards, hedgerows, groves, forest edges, forest minus forest edges, temporary grasslands and crops (Table A.1).

*2.2. Local land-use intensity index*

We defined local land-use intensity as an aggregation of three de-scriptors based on expert knowledge. We included defoliation (per-centage of grass defoliated during the year of sampling by mowing or grazing) (range = 100–300 %, mean = 201 %, standard deviation

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(SD) = 54 %), nitrogen fertilisation during the year of sampling (in-organic and organic fertilisation, animal deposition; Simon et al., 2000) (range = 0−100 kg N.ha−1. year−1, mean =27.3 kg N.ha −1. year−1, SD =37.5 kg N.ha−1. year−1), and earliness of the first defoliation event (degree days at the first mowing event during the sampling year, starting from 1 February and with a maximum value of 18 °C) (range = 922.0–1817.6 °C, mean = 1370.7 °C, SD = 227.6 °C; Table A.1). Information was obtained by questioning farmers. We used fuzzy inference systems (Mamdani and Assilian, 1975) to aggregate these three descriptors. Our final index ranged from 0 to 1 (mean = 0.44; SD = 0.35) after applying the methodology detailed in Appendix B.

*2.3. Soil characteristics*

For each transect on which pollinators were captured, we collected at three sampling point (beginning, middle, and end) three soil core samples per sampling point at a depth of 20 cm using an auger once during the study. We gathered the three soil core samples per sampling point in a single bulk sample. Seven soil variables were measured (in g.kg−1 of dry soil, except pH): phosphorus (P2 O5 with the Olsen-P method), total nitrogen and organic carbon (dry combustion), pH (di-luted in water), clay content, silt content, and sand content (five-frac-tion texture analysis after decarbonation). Data were averaged at the grassland scale (Table A.1). We performed principal component ana-lysis (PCA) on these soil variables with the FactoMineR package ([Lê](#page11) et al., 2008) of R software (R Core Team, 2019). The first axis of the soil PCA (explaining 65.8 % of the variance) included soil pH, total ni-trogen, organic carbon and texture (clay, silt and sand contents), which correlated strongly with each other. Hence, we could not distinguish these variables and kept coordinates of the first axis of the soil PCA in subsequent models (Borcard et al., 2011). The second axis of the PCA (explaining 17.1 % of the variance) was formed almost only by soil phosphorus fertility (Fig. C.1). Consequently, we kept raw phosphorus data in our models. The land-use intensity index was not correlated with the first axis of the soil PCA (r = -0.06,*P* = 0.817, df = 14) nor with phosphorus content (r = 0.41, *P* = 0.11, df = 14).

*2.4. Pollinator sampling*

From May-August 2017, we performed three (19 % of grasslands), four (25 % of grasslands) or five (56 % of grasslands) pollinator sam-pling sessions per grassland (10–17 May, 29 May-2 June, 8–15 June, 5–9 July and 6–8 August), yielding a total of 70 sampling events which are the minimum statistical unit in our study. The grasslands sampled were not mown for at least three weeks before sampling to allow plants to grow and bloom. During each sampling session, all grasslands ful-filling this prerequisite were sampled, with a maximum of seven days between sampling of the first and last grassland (except in one case). We divided each day into four time slots from 10:00-18:00. When possible, each grassland was randomly assigned to one of these time slots for each sampling session so that it was sampled at least once during each time slot. Sampling days were sunny without wind and with a temperature over 18 °C (Ebeling et al., 2008). We sampled pol-linators clearly foraging on flowers with a sweep net on one 400 m² transect (100 m long ×4 m wide) per grassland located in the centre of each grassland or the centre of a homogeneous vegetation station be-longing to the grasslands. Hence, we sampled flower visitors, but we termed them as pollinators due to their ability to transport pollen grains according to previous studies (Weiner et al., 2014). A single observer (J.G.) performed all sampling during a 15 min walk, without counting the time needed to process captured pollinators (i.e. the timer was stopped). Coleopterans belonging to family Nitidulidae and thrips (Thysanoptera) were not sampled because we did not know whether or how many of them were clearly interacting with a flower (Weiner et al., [2014](#page12)). Furthermore, their contribution to pollination function could be low except when they are super-abundant (Forup and Memmott, 2005).

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Pollination function was approximated as the interaction frequency of each sampling event (Bartholomée and Lavorel, 2019). At each sam-pling event, flower cover for each currently flowering species (except Poaceae and Juncaceae) was estimated as the mean percentage of cover (within 1 %) from seven 1 m² quadrats distributed randomly but reg-ularly along the entire transect.

*2.5. Floral traits*

*2.5.1. Cues and signals*

We did not incorporate in our study the phenotypic expression of floral traits of the same species. Hence, for each species, all floral traits were measured on individual plants sampled along different transects from different grassland and sampling events. For 47 of the 50 flow-ering plant species found, we measured the height of the top floral unit (FU) (i.e. aggregation of flowers accessible by a pollinator without flying) for 3–330 individuals (mean = 71) per species to the nearest cm using a tape measure. We also estimated FU area to the nearest mm using a calliper for the same individuals. We used the formula for a circular area (i.e. πr2) for rounded FU and that for a rectangular area (i.e. length × width) for other FU shapes (Fornoff et al., 2017). We also measured reflectance of flowers for 10 individuals per species (except *Veronica chamaedrys* with N = 4, *Campanula rapunculus* with N = 2, and *Crepis capillaris* with N = 1). We measured reflectance of leaves of themost abundant plant species (including Poaceae and Juncaceae, and representing 80 % of the total plant cover estimated in three 0.25 m² quadrats per grassland) before the first mowing event of each grassland to determine the colour background (Renoult et al., 2015). The method is detailed in **Appendix D**.

*2.5.2. Exploitation barrier*

We measured nectar tube depth for 10 individuals per species of a single flower per FU stored in 70° alcohol. Nectar tube depth is the distance between the nectaries and the entrance of a flower where only mouthparts can penetrate (Stang et al., 2006). It was measured to the nearest 0.01 mm with a stereomicroscope attached to a camera (Mo-ticam 5+, Motic, Xiamen, China). The entrance of flowers was de-termined by dissecting them and by observing pollinators interact with them directly in the field. For Asteraceae, the nectar tube entrance was described as before, but the nectar tube base began where the stiles no longer filled the tube. When nectaries were exposed directly to the air (e.g. Apiacae) we considered nectar tube depth equal to 0 mm ([Stang](#page11) et al., 2006). The same rule applied in absence of nectaries (e.g. *Plan-tago* spp.).

*2.5.3. Rewards*

In 2018, we estimated nectar sugar production per FU in 24 h fol-lowing Baude et al. (2016). We isolated FU from any interaction with pollinators with a net (mesh size 1 mm², F 510 net, Diatex, Lyon, France) for 24 h without precipitation for five individuals per plant species. Nectar was then sampled using microcapillary tubes (Micro-caps, Drummond Scientific, Broomall, PA, USA) with a calibrated ca-pacity of 0.5–5.0 μL. We measured nectar sugar concentration (g su-crose.100 g−1 solution) using hand-held refractometers adapted to low volumes (0–50 Brix and 45–80 Brix, Eclipse, Bellingham and Stanley, Tunbridge Wells, UK). We calculated sugar production by nectar per flower in 24 h (s; μg of sugar.flower−1.24 h−1) using the following formula:

s = 10dvC

with d the density of a sucrose solution at concentration C (g su-crose.100 g−1 solution) read from refractometers, and v (μL) the vo-lume of nectar produced by a single flower.

Density d was obtained using the formula d = 0.0037921C + 0.0000178C2 + 0.998860 (Corbet et al., 2001). Of the 50 plant species

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found in flower cover, we failed to sample 12 of them. We used data from the dataset of Baude et al. (2016) and Hicks et al. (2016). For one species, we used data for the species most closely related to it in our own dataset (*Medicago minima* for *Medicago lupulina*). We calculated sugar production by nectar per FU in 24 h by using the formula sFU = sN, with N the number of open flowers per FU counted on 10 in-dividuals per species. More details about the method are available in Appendix E.

*2.6. Floral trait indices*

*2.6.1. Floral trait community-structure indices*

Using the R package FD (Laliberté et al., 2014) and following the formula of Botta-Dukát (2005), we calculated, for each sampling event, Rao’s quadratic entropy index (FD*Q*) for all floral traits collected (FD*total*) except flower colour (see below) and for each floral trait se-parately (FD*traits*). FD*Q* describes functional entropy, and is correlated to both functional richness (i.e. volume of niche space occupied by species in a community) and functional divergence (i.e. how commu-nity abundance is distributed toward functional space extremities; [Mason et al., 2013](#page11)).

For flower colour, we calculated colour distances based on the *Apis* *mellifera* visual system and assembled them in a dissimilarity matrix,which was used to calculate FD*colour* (see **Appendix D** for details). As the dissimilarity matrix for FD*colour* was not based on the same method for measuring dissimilarity as the other traits, however, we could not include FD*colour* in FD*total* (Botta-Dukát, 2005). Finally, we calculated Community Weighted Means (CWMs) (e.g. Garnier et al., 2007) for each sampling event and each trait separately (except floral re-flectance).

Of the 50 species we sampled in our study, we were unable to measure flower area or height for three species, and flower colour for 10 species. Since these species had low abundance (flower cover of 0.14–4.17 %), we removed them from our analyses. Thus, the species abundance matrix contained 47 species for FD*total*, FD*traits* (except FD*colour)* and CWM*traits*, and 40 species for FD*colour*.

*2.6.2. Null models*

Flower species richness and Simpson’s diversity index were posi-tively correlated with FD*total* in our dataset (r = 0.47, *P* < 0.001 and r = 0.62, *P* < 0.001, respectively). To disentangle FD from taxonomic diversity, we created null models using the name-shuffling approach (Swenson, 2014). Briefly, the null models were calculated by re-calculating Rao's quadratic entropy index with species names shuffled in the trait matrix. This process was applied 9999 times to each plant community. Then, standardised effect sizes (SES) were calculated for each plant community using the formula:

*SES* .*FDQ = ObservdedFDQ*  *mean* (*nullmodelsFDQ* )



*sd* (*nullmodelsFDQ* )

A positive or negative SES is interpreted as an observed FD higher or lower, respectively, than the mean null value (Swenson, 2014). Our method for calculating null models standardised flower species richness and evenness, while functional differences between species were free to vary, providing information about FD independent of species richness and evenness. However, a relation between SES, flower species richness and evenness may be maintained if communities with the highest flower species richness are those with highest evenness and largest functional differences between species. This process was applied to FD*total* and to each FD*traits* separately. These SES.FD indices (hereafter called FD*total* and FD*traits* for simplicity) were used as FD indices in subsequent analyses.

*2.7. Statistical modelling*

We performed all analyses with R software and created all graphics with the R package gglplot2 (Wickham, 2016). All models were initially mixed models with grassland identity (grassland ID) and sampling session as random terms to account for spatial and temporal auto-correlation between statistical individuals. Mathematical equations of the models are available in Appendix F.

*2.7.1. Influences of local land-use intensification and soil characteristics on floral functional diversity*

Our first research question dealt with influences of local land-use intensification and soil characteristics on floral trait FD and CWM but also on total flower cover. Hence, we fitted 11 linear mixed models with the R package lme4. Response variables were FD*total*, FD*traits*, CWM*traits.* Explanatory variables were the same for all models: land use intensity index, the first axis of the soil PCA, phosphorus soil content, total crop cover, total SNH cover and overall habitat diversity, the three first terms depicting local environment factors and the three last ones depicting landscape factors.

*2.7.2. Relations between floral functional diversity and interaction frequency*

For our second research question, which dealt with relations be-tween floral functional diversity and interaction frequency, we fitted general linear mixed models with a negative binomial distribution to address overdispersion in the response variable (interaction frequency). We fit a model with FD*total* and total flower cover as explanatory variables and another one with FD*total* and flowering plant taxonomic richness as explanatory variables. Indeed, flower cover and flowering plant taxonomic richness showed a positive but saturating relation between them which prevented using them in the same model. Flower cover and flowering plant taxonomic richness showed a non-linear re-lation with interaction frequency. We modelled these relations with a second-degree polynomial regression. We chose a quadratic function because it allowed the best trade-off between modelling of the non-linear relations and ecological interpretation. To avoid correlation be-tween polynomial terms, orthogonal polynomial terms were calculated using the poly function in R. In two other models, we replaced FD*total* with all FD*traits*, and finally FD*total* with all CWM*traits*, with flower cover and flower cover^2 as co-variables.

*2.7.3. Model selection and validation*

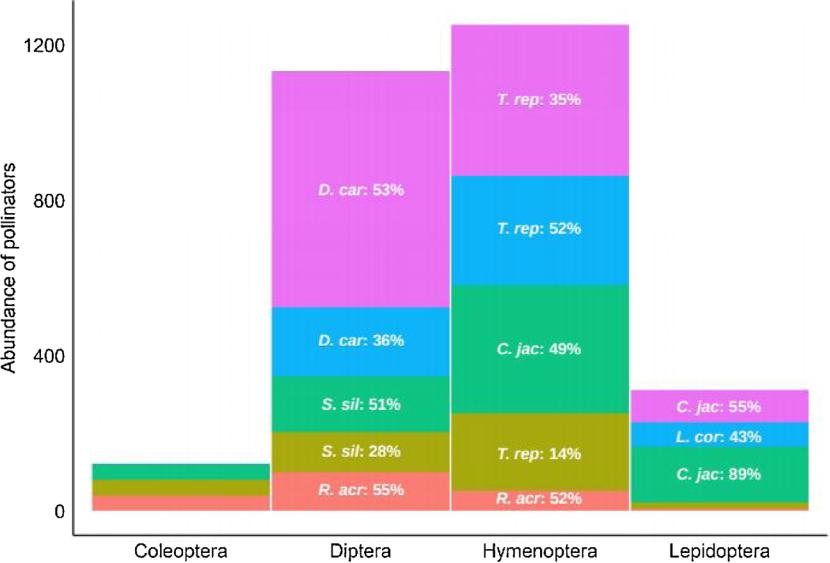
We standardised all explanatory variables (i.e. subtracting their mean and dividing by their standard deviation) due to large differences in scale between them. For all models, we considered a correlation coefficient (|r|) of 0.5 to be the threshold for collinearity and thus for variable selection (Dormann et al., 2013). When models showed sin-gular fit or failure to converge, we dropped the fixed or random term to allow for non-singular fit or avoid failure to converge (Barr et al., [2013](#page10)). We checked for model assumptions following Zuur and Ieno [(2016)](#page12). As we had low sample size (< 40) we selected models using AICc (Burnham et al., 2002). For nested models, when ΔAICc < 2, we could not distinguish which one explained interaction frequency best, so we kept the most parsimonious model. This approach can lead to model selection uncertainty (Burnham et al., 2011). However, alter-natives like model averaging are still in development (Dormann et al., [2018](#page10)), and can be harder to interpret.

We tested parameter significance for linear mixed models using t-tests based on Kenward-Roger’s methods with the R package lmerTest (Kuznetsova et al., 2017) and using Wald-tests for generalised linear mixed models with the R package lme4 (Bates et al., 2014). We cal-culated R² for final models using the R package MuMIn (Bartoń, 2019) and following Nakagawa and Schielzeth (2013), which provides in-formation about the variance explained by fixed effects for marginal models (hereafter R²m), and fixed and random effects for conditional

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**Fig. 1.** Abundance of the four main pollinator orders sampledin communities, by sampling session. Pink bars: mid-May; Mustard bars: early June; Green bars: mid-June; Blue bars: early July; Violet bars: early August. Percentage of interactions for the most frequent plant species in interaction with polli-nators per sampling session and per pollinator order are in-dicated (in white) only when interaction frequency exceeded



50. Hemiptera and Mecoptera are not represented. *R. acr*: *Ranunculus acris*; *S. sil*: *Silaum silaus*; *D. car*: *Daucus carota*; *T. rep*: *Trifolium repens*; *C. jac*: *Centaurea jacea*; *L. cor*: *Lotus cor-niculatus*. (For interpretation of the references to colour in thisfigure legend, the reader is referred to the web version of this article.)

models (hereafter R²c). We calculated pseudo-R² for generalised linear mixed models with the trigramma method due to their logarithmic link function. Finally, we assessed model quality using simulations by gen-erating model predictions that considered both fixed and random terms. We generated 200 predictions per model, which allowed them to tend toward the theoretical marginal distribution of the model’s dependent variable. Finally, we compared the theoretical marginal distribution to the distribution observed in our sample. We inspected model quality visually: if our sample overlapped the model predictions, we assumed a high goodness of fit.

**3. Results**

*3.1. General description of plant and pollinator communities*

We found 50 flowering plant species during our sampling events. Mean flowering plant species richness per community was 5.96 and ranged from 1 to 12 co-flowering species. Mean flowering Simpson’s diversity index was 0.58. We sampled a total of 2823 pollinators in 1065 minutes of sampling. Eight plant species represented more than 75

* of total interactions ([Fig. 1](#page5)): *Centaurea jacea* (17.1 % of total inter-actions), *Daucus carota* (15.0 %), *Trifolium repens* (13.2 %), *Silaum silaus* (8.2 %), *Ranunculus acris* (7.4 %), *Lotus corniculatus* (6.7 %), *Jacobea* *aquatica* (6.1 %) and *Trifolium pratense* (5.1 %). Of the pollinators, 1252were Hymenoptera (44.3 % of total interactions), with 505 *A. mellifera* (17.9 %) and 331 *Bombus* spp. (11.7 %). The second main pollinator order was Diptera (1132 individuals, 40.1 % of total interactions), with Muscidae (278 individuals, 9.9 %), Tachinidae (244 individuals, 8.6 %) and Syrphidae (239 individuals, 8.5 %) being the main families. We also sampled 311 Lepidoptera, most of which were *Maniola jurtina* (59.5
* of Lepidoptera), 122 Coleoptera, five Hemiptera and one Mecoptera. For most of the plant species encountered, we measured the fol-

lowing floral traits: height and area of the top FU, nectar tube depth and nectar sugar production, whose mean, sd and range values are

summarised in the Table 1. Flower colour, which was also measured, is not integrated in the Table 1 due to the high dimensionality of the raw data.

*3.2. Influences of land-use intensification and soil characteristics on floral trait FD and CWM*

We found no relation between local land-use intensification and floral trait FD (Fig. 2; Table 2). Among the landscape context variables, total crop cover had a negative influence on FD*sugar* (P = 0.03; R²m = 0.16; Table 2) and overall habitat diversity had a negative in-fluence on CWM*nectar tube* (P = 0.014; R²m = 0.11). We highlighted influences of soil characteristics on floral trait diversity. Total floral trait FD (FD*total*) increased with the first axis of the soil PCA and de-creased with phosphorus soil content (*P* = 0.031 and *P* = 0.029, re-spectively, R²m = 0.18, Fig. 2B and C). The same pattern was found for FD*area* (*P* = 0.016 and *P* = 0.010, respectively, R²m = 0.21, Fig. 2E and F), while FD*colour* only decreased with soil phosphorus content (*P* = 0.003, R²m = 0.18, Fig. 2L) and FD of nectar production per FU (FD*sugar*) only increased with the first axis of the soil PCA (*P*< 0.001, R²m = 0.16, Fig. 2Q). Among floral trait CWMs, only the CWM of flower area (CWM*area*) increased with the first axis of the soil PCA (*P* = 0.009, R²m = 0.10, Fig. G.1B). Finally, we found no influence of environmental variables on total flower cover ([Table](#page7) 2).

For models which contained fixed terms after model selection, the variance explained by fixed and random terms ranged from 0.27-0.46 (Table 2). When no fixed terms were kept in models after model se-lection, random terms explained at least one third of the variance (0.34-0.57), except for FD of nectar tube depth (FD*nectar tube*), for which variance explained by the random term was close to zero (R²c = 0.04). Our models showed moderate goodness of fit and residual hetero-scedasticity (Fig. H.1 and I.1), likely due to missing co-variables not measured in our study.

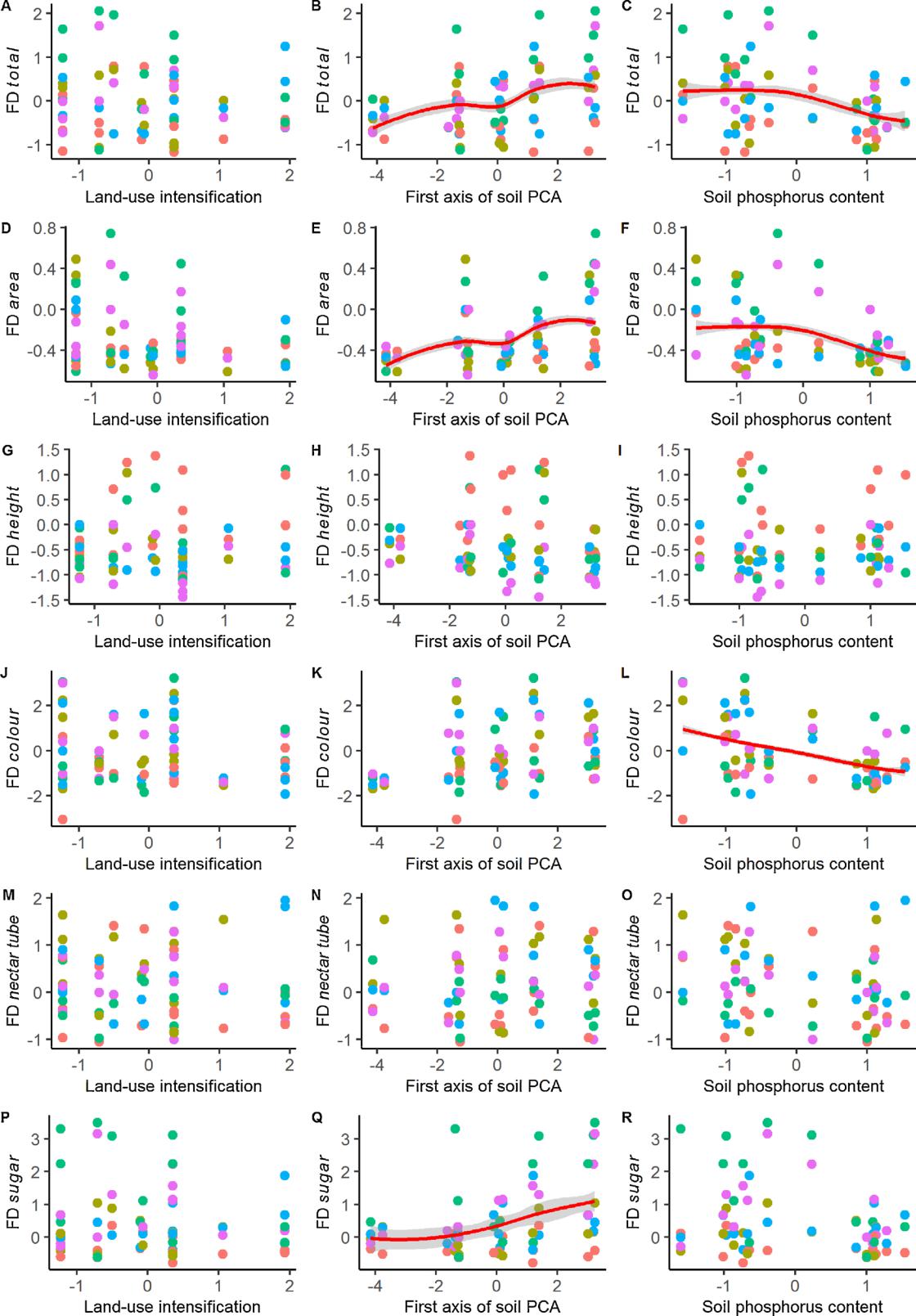
**Table 1**

Mean, sd and range of values of the floral traits measured. Flower colour is not integrated in the table.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Floral trait | Mean | Sd | Min |  | Max |  |  |
|  |  |  |  | |  |  |  |
| Height of the top FU (cm) | 38 | 15.23 | 13.98 (*Prunella vulgaris*) | | 76.95 | (*Anthriscus sylvestris*) |  |
| Area of the top FU (mm²) | 3.68 | 3.86 | 0.04 | (*Vicia hirsuta*) | 22.11 | (*Campanula rapunculus*) |  |
| Nectar tube depth (mm) | 2.3 | 2.67 | 0.00 | (15 different species including different Apiaceae, e.g.*S.* | 9.19 (*Rhinantus minor*) | |  |
| Nectar sugar production (μg of sugar.flower−1.24 h−1) | 912.09 | 1264.41 | *silaus*) | | 6209.48 (*Centaurea jacea*) | |  |
| 0.00 | (*Plantago lanceolata* and *Plantago media*) |  |

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**Fig. 2.** Predicted fit of linear mixed-effects models of the relation between floral trait functional diversity (FD) and local environment variables (soil characteristicsand land-use intensification index). Coloured dots are raw data categorized by sampling session. Pink dots: mid-May; Mustard dots: early June; Green dots: mid-June; Blue dots: early July; Violet dots: early August. Red lines: fitted values projected on raw data by considering other co-variables and random effects of final models, which explained the non-linear shape of curves. Grey shading around the red line: 95 % confidence intervals around smooth of predicted values. The absence of a red line indicates the absence of significant relation between the dependent and explanatory variable. Explanatory variables are standardized. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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**Table 2**

Results of linear mixed-effects models with floral trait indices explained by environmental variables.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Dependant variables | predictor | parameter estimate | standard error | t-value | *P* | variance of random term | R²m | R²c |  |
|  |  |  |  |  |  |  |  |  |  |
| FD*total* | Intercept | 0.005 | 0.137 | 0.036 | 0.973 |  | 0.18 | 0.29 |  |
|  | First axis of soil PCA | 0.083 | 0.038 | 2.208 | 0.031 |  |  |  |  |
|  | Soil phosophorus (g/kg) | −0.188 | 0.084 | −2.229 | 0.029 | 0.065 |  |  |  |
| FD*area* | 1|sampling date | −0.275 | 0.047 | −5.827 | 0.002 | 0.21 | 0.29 |  |
| Intercept |  |  |
|  | First axis of soil PCA | 0.038 | 0.015 | 2.476 | 0.016 |  |  |  |  |
|  | Soil phosophorus (g/kg) | −0.082 | 0.034 | −2.419 | 0.018 | 0.006 |  |  |  |
| FD*height* | 1|sampling date | −0.407 | 0.155 | −2.623 | 0.037 | 0.00 | 0.34 |  |
| Intercept | 0.086 |  |
|  | 1|sampling date |  |  |  |  |  |  |  |
| FD*colour* | 1|grassland ID |  |  |  | 0.707 | 0.049 | 0.18 | 0.28 |  |
| Intercept | −0.596 | 0.171 | −3.478 |  |  |
|  | Soil phosophorus (g/kg) | 0.003 | 0.047 |  |  |  |
|  | 1|sampling date |  |  |  |  |  |  |  |
| FD*nectar tube* | 1|grassland ID | 0.214 | 0.113 | 1.886 | 0.118 | 0.139 | 0.00 | 0.04 |  |
| Intercept | 0.022 |  |
| FD*sugar* | 1|sampling date |  |  |  | 0.137 | 0.16 | 0.46 |  |
| Intercept | 0.435 | 0.097 | 4.494 |  |  |
|  | First axis of soil PCA | 2.94E-05 |  |  |  |  |
|  | Total crop cover (%) | −0.210 | 0.097 | −2.176 | 0.033 | 0.314 |  |  |  |
| CWM*area (cm²)* | 1|sampling date | 3.596 | 0.309 | 11.625 | 8.88E-05 | 0.10 | 0.27 |  |
| Intercept |  |  |
|  | First axis of soil PCA | 0.212 | 0.070 | 3.034 | 0.009 | 0.358 |  |  |  |
|  | 1|sampling date |  |  |  |  |  |  |  |
| CWM*height (cm)* | 1|grassland ID | 36.758 | 2.182 | 16.850 | 3.15E-06 | 0.018 | 0.00 | 0.44 |  |
| Intercept | 19.299 |  |
|  | 1|sampling date |  |  |  |  |  |  |  |
| CWM*nectar tube (mm)* | 1|grassland ID | 2.913 | 0.378 | 7.711 | 2.36E-04 | 6.664 | 0.11 | 0.45 |  |
| Intercept |  |  |
|  | Overall habitat diversity | −0.490 | 0.177 | −2.768 | 0.014 | 0.558 |  |  |  |
|  | 1|sampling date |  |  |  |  |  |  |  |
| CWM*sugar (μg of sugars.FU-1.24h-1)* | 1|grassland ID | 1407.900 | 325.300 | 4.328 | 0.005 | 0.212 | 0.00 | 0.50 |  |
| Intercept | 430991.000 |  |
|  | 1|sampling date |  |  |  |  |  |  |  |
| Total flower cover | 1|grassland ID | 0.174 | 0.042 | 4.119 | 0.005 | 171896.000 | 0.00 | 0.57 |  |
| Intercept | 0.007 |  |
|  | 1|sampling date |  |  |  |  |  |  |  |
|  | 1|grassland ID |  |  |  |  | 0.004 |  |  |  |
|  |  |  |  |  |  |  |  |  |  |

*3.3. Influences of floral trait FD, CWM and flower cover on interaction frequency*

We found a positive relation between interaction frequency and FD*total* (*P* = 0.002, Fig. 3A). For the FD of each trait separately, we found an increase in interaction frequency only with FD*sugar* (*P* < 0.001, Fig. 3B). We found no relation between floral trait CWMs and interaction frequency. Second-degree polynomials explained the rela-tion between interaction frequency and total flower cover (Fig. 3C, Table 3) and that between interaction frequency and flowering plant species richness (Fig. 3D, Table 3). R²m of the models ranged from 0.31 to 0.43 (Table 3) and showed a high goodness of fit (Fig. H.1).

**4. Discussion**

*4.1. Influences of local environmental variables on floral functional diversity*

In this study, we found influences of soil characteristics on floral functional diversity. We found a negative influence of soil phosphorus content on total floral trait FD (FD*total*), and more specifically on FD of both flower colour and flower area. Soil phosphorus content is well known to influence plant species diversity and can be related to many factors including past intensification (e.g. phosphorus from inorganic fertilisation, livestock manure and slurry spreading, Ceulemans et al., [2014](#page10)). Along a gradient of soil phosphorus content, plant species di-versity has been shown to decrease in European grasslands ([Ceulemans](#page10) et al., 2014), but not at a more global scale (Soons et al., 2017). Ceulemans et al. (2013) observed that competitive plant species

following the Competition-Stress-Ruderal (C-S-R) Grime classification (e.g. Grime, 2007) showed a preference for high soil phosphorus con-tent.

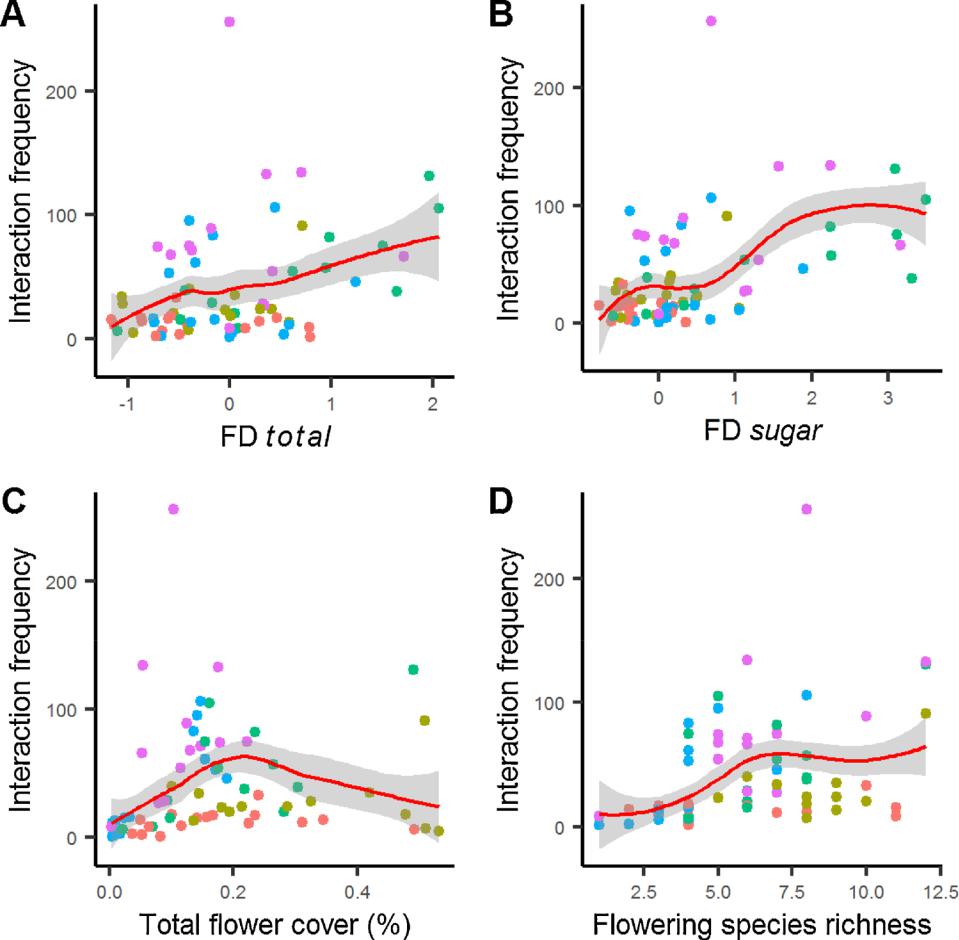
A same mechanism could explain our results. Indeed, part of grasslands in our study (Fig. J.1) had more than 0.05 g.kg−1 of soil phosphorus content (i.e. the optimum for plant nutrition; [Janssens](#page11) et al., 1998). Decrease in floral functional diversity would thus be due to more light competition on grasslands with high soil phosphorous content, leading to competitive exclusion and a decrease in plant functional diversity (Helsen et al., 2014). However, we expected a de-crease in both FD*total* and the FD of each trait separately, which was not the case for flower height, nectar tube depth or flower sugar (Fig. 3I, O and R). Flower height could have more phenological plasticity than other floral traits we measured, which would buffer differences be-tween communities. Considering phenotypic variation could improve estimates of height FD. The absence of a significant influence of phos-phorus content on nectar tube depth FD could have been due to how we measured nectar tube depth for Asteraceae, which could also buffer differences between communities. Finally, more studies are needed to cover a wider range of phosphorus soil contents; our grasslands had a soil phosphorus content of 0.01–0.06 g.kg−1, which represents a small gradient ([Ceulemans et al., 2014](#page10)).

This result could have direct implications for grassland restoration which can imply addressing the high phosphorus contents in grassland soil. Soil phosphorus content, which can result from past PK fertilisa-tion, greatly influence the flowering plant species which can maintain stable populations without sowing in restored ecosystems (Pywell et al., [2007](#page11); Kirkham et al., 1996), and potentially floral functional diversity as we highlighted in this study. Consequently, our results bring new

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**Fig. 3.** Predicted fit of general linear mixed-effects models of the relation between inter-action frequencies and A) FD*total*, B) FD*sugar*, C) Total flower cover and D) Flowering plant species richness. A) and C) are generated by the same model. Coloured dots are raw data categorized by sampling session. Pink dots: mid-May; Mustard dots: early June; Green dots: mid-June; Blue dots: early July; Violet dots: early August. Red lines: fitted values projected on raw data by considering other co-variables and random effects of final models, which ex-plained the non-linear shape of curves for A and B. Grey shading around the red line: 95 % confidence intervals around smooth of pre-dicted values. (For interpretation of the refer-ences to colour in this figure legend, the reader is referred to the web version of this article.)



elements supporting soil phosphorus analyses for targeting restoration sites, as commonly used in United-kingdom agri-environment schemes (Higher Level Stewardship).

We also found a positive influence of an association of soil pH, soil nitrogen content and soil texture on floral trait FD (FD*total*) and more specifically on FD of both flower area and flower sugar. Our main hy-pothesis laid on soil nitrogen fertility, but we could not distinguish its influence from those of pH and soil texture. However, declining

belowground stress (i.e. an increase in fertility in our case) should lead to more FD in traits associated with soil-resource use (Mason et al., [2013](#page11)), until reaching levels at which exclusive competition occurs, which could in turn influence floral trait FD. The positive influence of the first axis of the soil PCA on flower area CWM may be explained by the selection of plant species with higher investment in visual attrac-tiveness to pollinators due to more resource availability. Our study may confirm the overall negative influence of phosphorus fertility on plant

**Table 3**

Results of generalised linear mixed-effects models, with interaction frequency explained by total floral trait functional diversity (FD) (FD*total*), total flower cover, flowering plant species richness and FD of sugar production per floral unit (FD*sugar*). Models with community-weighted means are not presented because no fixed effects were kept in them.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Dependant variables | predictor | parameter estimate | standard error | z-value | *P* | variance of random term R²m | R²c | df | AICc |  |
|  |  |  |  |  |  |  |  |  |  |  |
| Interaction frequency | Intercept | 3.386 | 0.294 | 11.525 | < 2E-16 | 0.35 | 0.63 | 64 | 620.143 |  |
|  | FD*total* | 0.300 | 0.095 | 3.160 | 0.002 |  |  |  |  |  |
|  | Total flower cover | 3.324 | 0.850 | 3.912 | 9.16E-05 |  |  |  |  |  |
|  | Total flower cover^2 | −4.026 | 0.753 | −5.346 | 9.01E-08 | 0.399 |  |  |  |  |
|  | 1|sampling date |  |  |  |  |  |  |  |  |
|  | Intercept | 3.418 | 0.223 | 15.310 | < 2E-16 | 0.31 | 0.48 | 64 | 630.169 |  |
|  | FD*total* | 0.265 | 0.105 | 2.523 | 0.012 |  |  |  |  |  |
|  | Flowering species richness | 4.029 | 0.869 | 4.638 | 3.51E-06 |  |  |  |  |  |
|  | Flowering species richness^2 | −2.023 | 0.799 | −2.532 | 0.011 | 0.211 |  |  |  |  |
|  | 1|sampling date |  |  |  |  |  |  |  |  |
|  | Intercept | 3.364 | 0.264 | 12.730 | < 2E-16 | 0.43 | 0.65 | 64 | 614.243 |  |
|  | FD*sugar* | 0.407 | 0.101 | 4.027 | 5.64E-05 |  |  |  |  |  |
|  | Total flower cover | 3.391 | 0.814 | 4.165 | 3.11E-05 |  |  |  |  |  |
|  | Total flower cover^2 | −4.116 | 0.727 | −5.664 | 1.48E-08 | 0.318 |  |  |  |  |
|  | 1|sampling date |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |

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species co-existence (Ceulemans et al., 2014), while other soil char-acteristics could be at the lower range of the belowground stress gra-dient.

During this study, local land-use intensification was not the main driver of floral functional diversity which may be explained by the small gradient of local land-use management. We observed high cor-relation (r = 0.6, P < 0.05) between defoliation regime (proxy of mortality) and nitrogen fertilisation (proxy of productivity) in our grasslands which could lead to no difference in plant diversity between our plots according to the dynamic equilibrium model (Huston, 2014). Decorrelation between defoliation regime and fertilisation could be greater with a larger local intensification gradient, which could lead to a negative influence of intensification on floral trait FD, as found, for instance, by Binkenstein et al. (2013). The same reasons could explain the absence of influence of land-use intensification on CWMs or total flower cover, though other authors did find an influence (e.g. negative influence on CWM*height* by Garnier et al., 2007; negative influence on forb abundance by [Pakeman et al., 2017](#page11)).

None of the relation between landscape context and floral func-tional diversity were significant, except total crop cover which had a negative influence on the diversity of sugar production, and overall habitat diversity which had a negative influence on mean nectar tube depth. Crops may have lower floral functional diversity than other habitat which were considered in our study. Hence, in comparison with grassland with a landscape dominated by other habitats, it could lower the functional diversity of plants able to colonise grassland. Negative influence of overall habitat diversity on mean nectar tube depth is more surprising. It might mirror an indirect effect of permanent grassland cover. Indeed, there is a negative correlation between overall habitat diversity and permanent grassland cover (r = -0.56, *P* < 0.001). Permanent grassland may be covered by plants with deeper nectar tube depth, like Fabaceae, than other habitat. Hence, an increase in overall habitat diversity could lead to a decrease in the mean nectar tube depth of plants able to colonise grassland at the landscape scale. Future analyses would provide a better understanding of why this trait is in-fluenced and the other traits studied here are not.

*4.2. Influences of floral functional diversity on interaction frequency*

In support of our second hypothesis, we found for the first time a positive relation between floral trait FD and interaction frequency, in-dependent of flowering plant species richness. This result is a new element of the positive biodiversity-ecosystem functioning framework. Furthermore, while pollinators face a global decline (Potts et al., 2010), our results highlight that, besides plant species richness (e.g. [Ebeling](#page10) et al., 2008), floral trait FD could favour pollinator abundance. Hence, management practices which increase floral trait FD could be a new way to improve grassland quality for pollinators. For instance, it could drive species composition in seed mixes sown in grassland. A future step could be to integrate positive relation between floral trait FD and in-teraction frequency with grassland productivity (biomass and quality; Poutaraud et al., 2017) and production resilience. Studying these po-tential trade-offs should provide more information about how farm-lands can balance human food needs and biodiversity conservation is-sues ([Burkle et al., 2017](#page10)).

However, our results contradict those of Fornoff et al. (2017) and Uyttenbroeck et al. (2017), who showed a negative influence of floral trait FD on interaction frequency in two European experimental sites. This contradiction may be explained by differences in the diversity (richness and evenness) of pollinator communities sampled in each study. Fornoff et al. (2017) sampled only 67 pollinator species, of which 62 % were *A. mellifera*, while Uyttenbroeck et al. (2017) sampled 68 species, of which 39 % were *Eristalis tenax* (Syrphidae) and *A. mellifera*. In contrast, we sampled more than 200 species. As their sampling effort was similar to or much higher than ours, the low diversity of pollinators they found could have been due to the locations of their experimental

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sites, whereas we sampled a landscape dominated by semi-natural ha-bitats.

We can also explain this positive relation by the fact that increased pollinator diversity combined with increased floral trait FD could po-tentially lead to an increase in niche partitioning (e.g. Junker et al., [2015](#page11)). This could lead to an increase in interaction frequencies, with a subsequent increase in efficiency (e.g. in flower handling or sugar ex-traction rate, due to better trait matching between nectar tube depth and mouthpart length; Klumpers et al., 2019). However, when we modelled interaction frequency using a combination of all floral trait FD, we found a positive relation only with diversity of sugar production per FU. This suggests that an increase in reward production diversity might be a main factor for niche partitioning of pollinators, probably according to their metabolic needs (Vaudo et al., 2015); however, dif-ferences between metabolic needs of pollinators still need to be con-sidered to validate this hypothesis. One could then relate this pollinator trait to the nectar sugar production of the flowers they visit. Finally, niche partitioning according to sugar production might also reflect the degree of sociality of the pollinators we sampled. For instance, social pollinators may focus on flowers that produce more rewards if they must harvest nectar for themselves and their colony. These social in-sects may also occupy the best nectar resources due to their high den-sities and ability to communicate resource positions, forcing other species to exploit less profitable resources (Henry and Rodet, 2018). Overall, pollinators that forage to feed their offspring (i.e. bees, in our study) can be expected to visit flowers that provide more rewards than pollinators with free-living larvae.

The absence of relation between interaction frequency and other floral trait FD was not expected. For instance, flower colour is a major trait in niche partitioning according to Junker et al. (2013). Further-more, Renoult et al. (2015) found a positive relation between specia-lisation and matching of flower colour to pollinator visual systems in grasslands. An absence of niche partitioning according to flower colour may be possible if pollinators use plasticity and associative learning when they process flower colour (van der Kooi et al., 2019). Concerning the diversity of nectar tube depth, we may have obtained a low di-versity of mouthpart length in our sample despite its high diversity of pollinators. Indeed, approximately half of the pollinators we sampled were Diptera, specifically Muscidae and Tachinidae. While some Dip-tera families have long mouthparts (e.g. Empididae), Diptera are often considered to have short mouthparts (Woodcock et al., 2014b). Fur-thermore, most of the Diptera we sampled have sponging-sucking mouthparts which are well adapted for exploiting viscous nectar from shallow flowers ([Krenn et al.,](#page11) 2005; [Woodcock et al., 2014b](#page12)).

We found no relation between floral trait CWMs and interaction frequency, which is not in agreements with previous results. [Fornoff](#page10) et al. (2017) and Hegland and Totland (2005) found a positive relation between interaction frequency and CWM*area* and interaction fre-quency. Fowler et al. (2016) highlighted a positive relation between mean sugar produced per flower and number of visits by *Bombus* spp. We can explain this absence of relation in our study by a greater role of diversity of sugar production than of its mean value, probably due to a high diversity of pollinator metabolic needs.

*4.3. Relations between total flower cover and interaction frequency*

We highlighted a non-linear relation between total flower cover and interaction frequency, while a linear relation is commonly found in previous studies (e.g. Ebeling et al., 2008; but see Venjakob et al., [2016](#page12)). Consequently, we confirmed in part our hypothesis of an in-crease in interaction frequency when attractiveness and reward avail-ability increase. In our study, we could explain the negative relation between total flower cover and interaction frequency when total flower cover exceeded 30 % by considering plant species composition. Indeed, most high flower cover was due to plants with low attractiveness to pollinators (e.g. *Galium mollugo*) or even pollen toxicity (e.g. *R. acris*;

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[Praz et al., 2008](#page11)).

We also found a non-linear relation between interaction frequency and flowering plant species richness, with a negative relation when richness exceeded approximately six co-flowering species. Ebeling et al. [(2008)](#page10), with the same range of flowering plant species, found only a positive relation between flowering plant species richness and inter-action frequency. In our study, 13 of the 27 plant communities with more than six flowering plant species were dominated by *G. mollugo* or *R. acris*. Consequently, we may not have studied some floral traits withstrong influence on defining niche partitioning, such as volatile organic compounds or pollen quality (Junker and Parachnowitsch, 2015), which could explain the non-linear relations we found for total flower cover and flowering plant species richness. More studies are needed to know whether these non-linear relations are due only to these specific species or to a more general pattern (e.g. resulting from a trade-off between attractiveness and dominance).

**5. Conclusion**

In this study, we showed how soil characteristics, and more parti-cularly phosphorus content, can influence floral functional diversity. This result, if generalised to a larger set of grasslands, could have direct implications for grassland management and restoration. We also found a positive influence of floral functional diversity on plant-pollinator interaction frequency, due mainly to the functional diversity of nectar sugar provided by plant communities. Our results challenge previous studies but agree with known relations between biodiversity and eco-system functioning. However, the cause of this positive relation still needs to be validated, notably by considering pollinator matching traits associated with sugar production, such as pollinator metabolic re-quirements (approximated by body mass) and degree of sociality. Finally, non-linear relations between total flower cover and interaction frequency may reveal a trade-off between flower abundance and at-tractiveness. Future studies will need to consider floral traits that are more difficult to measure (e.g. secondary metabolites in pollen) to in-vestigate this potential trade-off.

**Authors’ contributions**

JG, AM, SP and CR conceived the idea. JG designed the study, ad-vised by AM. JG led the writing of the manuscript and collected the data. JV and SP developed the intensification index. JG, MT and MD analysed the data. JG, AM, MB and CR interpreted the results. All au-thors contributed critically to the drafts and gave final approval for publication.

**Declaration of Competing Interest**

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

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**Appendix A. Supplementary data**

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.agee.2020.107033>.

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