**Prospective titles:**

Biological correlates and ecological drivers of seed oil content in alpine species

Unravelling seed oil content patterns in alpine species

**Authors:** C. Espinosa del Alba1,2, A. Mondoni3, Silvia Baizán, E. Fernández-Pascual1,2 & B. Jiménez-Alfaro1,2.

**Affiliations:**

1-Biodiversity Research Institute (IMIB), University of Oviedo-CSIC-Principality of Asturias, Mieres, Asturias, 33600 Spain.

2-Department of Organismal and systems Biology, University of Oviedo, Oviedo, Asturias, 33071 Spain

3-Dipartimento di Scienze della Terra e dell’Ambiente, University of Pavia, 27100 Pavia, Italy.

**Correspondence author**: Clara Espinosa del Alba. Email: clara.esdal@hotmail.es

**ORCID**

Clara Espinosa del Alba. https://orcid.org/0000-0001-8634-5808

Andrea Mondoni. <https://orcid.org/0000-0002-4605-6304>

Eduardo Fernández Pascual. <https://orcid.org/0000-0002-4743-9577>

Borja Jiménez-Alfaro. <https://orcid.org/0000-0001-6601-9597>

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**Data availability statement**

Raw data and R script for analysis are available in GitHub, once the manuscript is accepted Github repository will be public and stored in Zenodo.

**Author contributions**

Conceptualization: Clara Espinosa del Alba, Borja Jiménez-Alfaro; Investigation: Clara Espinosa del Alba, Silvia Baizan, Andrea Mondoni; Formal Analysis: Clara Espinosa del Alba; Writing – original draft: Clara Espinosa del Alba; Writing – Review & Editing: all authors; Supervision: Borja Jiménez-Alfaro, Eduardo Fernández Pascual, Andrea Mondoni; Funding acquisition: Borja Jiménez-Alfaro.

Abbreviations:

UFA: Unsaturated Fatty Acids; SFA: Saturated Fatty Acids; FAME: Fatty Acid Methyl Esters

## Abstract (297 words)

Seed oil content, one of the crucial energy reserves in angiosperms, is important for a wide range of biological processes that influence plant fitness. Seed oil can be considered an adaptive trait with potential trade-offs involving other seed traits and displaying latitudinal patterns. Additionally, seed oil composition, namely the relative proportions of saturated and unsaturated fatty acids, has been proposed as a key trait for micro- and macro-evolutionary biogeographical patterns. However, the availability of oil data for wild species is minimal, and few studies have addressed the potential biological and ecological correlates of seed oil content and composition.

Our study aims to explore these relationships with a unique data set of 47 European alpine species co-occurring in the Cantabrian Mountains (NW Spain) across altitudinal and topographical gradients. Seed oil content and composition analyses were conducted with fresh seeds collected in natural communities, successfully detecting 26 fatty acids. We also measured seed traits with ecological and evolutionary relevance, such as seed mass, seed longevity, and germination timing. Species local ecological optimum along microenvironmental gradients were calculated based on 160 community plots sampled in the study area.

We found no relationships between seed oil content and composition with seed mass and germination timing, but we did find evidence that oil content and composition significantly influence seed longevity. These results are essential for understanding the potential impacts on soil seed banks and for adapting conservation strategies for seed banks and/or restoration programmes. Interestingly, and against expectations, we did not find patterns corroborating regional or local gradients driving changes in seed oil content or composition. The lack of significant patterns might indicate strong phylogenetic constraints on oil content. This is a meaningful advance in providing a more complete picture of the correlates of oil content and oil composition for wild species.

Keywords: Seed oil content; Seed oil composition; Germination; Longevity; Alpine species; altitudinal gradient; microenvironmental gradients.

## 1. Introduction (1297 words)

Seeds are energy reservoirs, accumulating three main macromolecules: proteins, lipids and carbohydrates (Levin, 1974; Baud and Lepiniec, 2010). The quantity and quality of these reserves are directly related to plant fitness, as they influence germination success (Linder, 2000), seedling establishment (Graham, 2008) and seed viability (Hoekstra, 2005). In angiosperms, the predominant reserve forms are lipids and carbohydrates, the firsts majorly in the form of triglycerides (an ester of glycerol plus three fatty acids) (Harwood, 1980; Voelker and Kinney, 2001), and the latter accumulated as starch (Levin, 1974). Carbons in fatty acids, through lipid oxidation, release more than twice as much energy as the oxidation of starch on a per g basis of dry weight because their carbons are highly reduced (Levin, 1974; Baud and Lepiniec, 2010). Consequently, plants mainly rely on lipids (hereafter “seed oil”) for energy mobilization during germination (Harwood, 1980). In endospermic seeds, seed oil is mainly stored in the cotyledons and endosperm (Ellis, 2006) but also in the radicle and hypocotyl (Li *et al.*, 2006). Seed oil content is highly constrained by phylogeny and subject to evolutionary change (Levin, 1974), but variation also exists within and among genera of the same family (Levin, 1974; Bretagnolle *et al.*, 2016).

In seeds, most fatty acids range from 10 to 22 carbons in length, and the carbons may be joined by single or double bonds, referred to as saturated (SFA) and unsaturated fatty acids (UFA), respectively (Ellis, 2006). The relative proportion of specific fatty acids and the relative proportions of unsaturated and saturated fatty acids constitute what we will refer to as “oil composition”. The most abundant fatty acids found in seeds are the unsaturated oleic acid (18:1n-9), linoleic acid (18:2n-6), alpha-linolenic acid (18:3n-3), and saturated palmitic (16:0) and stearic (18:0) acids (Voelker and Kinney, 2001; Ellis, 2006; Baud and Lepiniec, 2010). Unsaturated and saturated fatty acids possess distinct biochemical properties (Lehninger, Nelson and Cox, 1993), influencing their functioning as an energy reserve. On a per-carbon basis, unsaturated fatty acids are more expensive to produce and yield less energy when oxidised than saturated fatty acids (Lehninger, Nelson and Cox, 1993; Linder, 2000). Moreover, unsaturated fatty acids have a higher potential for oxidative damage (Rael et al., 2004), and so they require antioxidant molecules to prevent damage. Hence, an optimal energy storage strategy for seeds should maximise saturated storage instead of unsaturated fatty acids (Linder, 2000). However, the relative abundance of unsaturated and saturated fatty acids varies significantly in angiosperms (Voelker and Kinney, 2001), and contrary to expectations, many species synthesise a low amount of saturated fatty acids (Linder, 2000). A potential explanation for this anti-intuitive pattern is that saturated fatty acids have a higher melting point than unsaturated fatty acids (Knothe and Dunn, 2009; Sanyal and Decocq, 2016), making energy less accessible at low temperatures. Additionally, the biochemical properties of unsaturated fatty acids increase the unpalatability, serving as a defence against herbivory, and also function as anti-freezing compounds (Linder, 2000); see Figure 1 for a summary table.

Seed oil content and composition influence a wide range of biological functions and, as such, are crucial for plant adaptation (Levin, 1974; Sanyal and Decocq, 2016). It is known that seed energy and oil content are positively correlated (Levin, 1974; Bretagnolle et al., 2016). Such a relationship suggests that oil synthesis is energetically costly and could be advantageous only for small seeds, which can store more energy in a smaller volume (Bretagnolle et al., 2016). Large seeds generally store less oil than small seeds; however, a tiny oily seed can release as much energy as a larger, starchy seed (Bretagnolle et al., 2016). There is a consensus in the literature that seed mass and oil content are negatively correlated, with seed oil content contributing up to 60% of seed mass weight (Ellis, 2006; Theodoulou and Eastmond, 2012). In concordance with the correlations reported, selection could act simultaneously on multiple seed traits (Sanyal and Decocq, 2016). Previous studies have also claimed that seed oil content influences seed longevity (Hoekstra, 2005), meaning the ability of seeds to remain viable over specific storage periods, which determines the capacity of seeds to persist in the soil seed bank. Secondary lipid oxidation metabolites, needed for energy release, can cause detrimental effects on other macromolecules (Graham, 2008). Remarkably, the oxidation of unsaturated fatty acids contributes significantly to producing free radicals and subsequent attacks by these radicals (Priestley and Leopold, 1979). To prevent deleterious effects, oily seeds also store antioxidants, and studies show a positive correlation between antioxidant levels and the relative proportion of unsaturated fatty acids (Sattler et al., 2004). Accordingly, previous studies have found oily seeds to be more prone to ageing (Nagel and Börner, 2010; Neto et al., 2019), and detailed studies, including oil composition, have also shown that a higher proportion of unsaturated fatty acids is associated with shorter longevity (Hoekstra, 2005). Thus, not only oil content but also oil composition affects the storage behaviour of seeds (Volk et al., 2006). Further investigations on the effects of seed oil content and composition on longevity are desirable (Pritchard and Dickie, 2003) but are lacking for most wild species.

In a pioneering study on wild species, Levin (1974) found significant seed oil content variations depending on habitat type (oil increased in woodlands) and life form (lower oil in herbs than in shrubs and trees) but no geographical differences between temperate, subtropical and tropical regions. However, a more recent study found a significant latitudinal gradient with higher seed oil content in tropical plants compared to temperate plants (Sanyal and Decocq, 2016), probably because, in the tropics, seeds need higher energy to survive the first life stages, with high competition for light (Levin, 1974). In the same study, they also found significant oil composition gradients, with saturated fatty acids decreasing by 0.1% for each degree of latitude (Sanyal and Decocq, 2016), in concordance with previous studies (Linder, 2000). The different fatty acids' melting points could explain this pattern. At higher latitudes (cooler temperatures), seeds with higher unsaturated fatty acids proportions, which have a lower melting point, can access the energy stored and germinate earlier, providing a competitive advantage (Linder, 2000). At lower latitudes (higher temperatures), seeds do not have a temperature limitation to access higher saturated fatty acids energy resources with higher melting points (Sanyal and Decocq, 2016). Higher growing temperatures have also been correlated with higher oil content in several cultivars (Piper and Boote, 1999) and higher saturated fatty acids accumulation (Sanyal and Linder, 2013). Consequently, seed oil content and composition seem to be under strong selection by temperature at both micro- and macro-ecological levels (Sanyal and Linder, 2013; Sanyal and Decocq, 2016).

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Figure 1. A) Fatty acid biochemical properties. B) Altitudinal oil content and unsaturated UFA/SFA ratio (Unsaturated Fatty Acids /Saturated Fatty Acids) patterns expected in our study.

In this study, we investigate biological correlates and ecological drivers of seed oil content and composition in a regional alpine flora. Alpine ecosystems are unique laboratories to explore seed oils because they are subjected to strong ecological filters towards similar life forms (Körner, 2021), but at the same time, support high evolutionary diversity (REF Testolin?) and functional variation across topographical gradients (Scherrer and Körner, 2011). Also, alpine plants have adapted to multiple reproductive strategies (through different flowering, seed and clonal traits); they may have responded to elevation and microclimatic snow gradients with a specific combination of seed oil content and composition. Alpine species are generally known for having average-sized seeds (Körner, 2021), short longevity (Mondoni *et al.*, 2011), and delayed germination phenology due to physiological dormancy (Fernández-Pascual et al., 2021; Espinosa del Alba, Fernández-Pascual and Jiménez-Alfaro, 2024). We expect seed mass, longevity, and earliness of germination (i.e. environmental heat sum (EHS)) to correlate with oil content and composition; see Table 1 for detailed expectations. We also expect to find regional and local oil patterns related to temperature along an altitudinal gradient, in concordance with the results previously reported for the latitudinal gradient (Sanyal and Linder, 2013; Sanyal and Decocq, 2016); see Figure 1 and Table 1 for detailed expectations.

Table 1. Study expectations relationships between seed oil content and composition with biological and ecological correlates.

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | **Oil content** | **Ratio UFA/SFA** |
| Biological correlates | |  |  |
|  | Seed mass | Negative | Negative |
| Seed longevity | Negative | Negative |
| Earliness of germination | Positive | Positive |
| Local ecological drivers | |  |  |
|  | Growing Degree Days | Positive | Negative |
|  | Freezing Degree Days | Negative | Positive |
|  | Snow Days | Positive | Positive |

## 2. Materials and Methodology (1626 words)

### Study system

We focused on herbaceous alpine species from grassland communities in the Cantabrian Mountains (north-western Spain), a transitional mountain system between the temperate and Mediterranean regions in southern Europe (García-Gutiérrez *et al.*, 2018). These grassland communities are continuously distributed along the mountain range, occupying reduced areas above the treeline and around mountaintops between 1750 and 2500 m a.s.l (González Le Barbier et al., 2025). Grassland communities are predominantly dominated by Poaceae and Cyperaceae, and the main life forms are hemicryptophytes and chamaephytes. Climatic conditions follow a north-south temperature and precipitation gradient, with colder and wetter conditions on northern slopes compared to warmer and drier conditions on southern slopes. Mean annual temperatures in the study area range from 2.5 to 4.5 ºC and mean summer precipitation ranges from 160 to 260mm (values extracted from Chelsa 2.1 bio1 and bio17, Karger *et al.*, 2017). We established eight sampling sites in the alpine grassland zone (above 1900 m a.s.l.), four on the northern slopes and four on the southern slopes of the Cantabrian Mountains, ensuring representation of the flora from the two biogeographical regions. We collected floristic and community composition data for all vascular plants at each sampling site, and classified species as strict alpine or generalist according to their altitudinal distribution in the study region. We identified plant specialists as those significantly associated with alpine grasslands, using the Indicator Values (IndVal) in the indicspecies R package (De Cáceres and Legendre, 2009). The calculations were based on 12,000 vegetation plots of grasslands stored in the SIVIM database (www.sivim.org) for the ecoregion in which our system occurs (The WWF Cantabrian Mixed Forest ecoregion). Seed collection permits were granted for the sampling sites inside Picos de Europa National Park: expedient numbers CO/09/203/2021 and CO/09/192/2023.

### Seed oil and seed mass

In a preliminary exploratory analysis, we tested a destructive method to calculate seed oil content and composition for three seed lots from five species and different taxonomic families. Since we didn´t find significant differences within the seed lots, we focused our seed collections on a single sample per species. Out of the 119 initially recorded species, we collected enough material for 47 species (19 families) with a minimum of 200 mg of dry seeds per sample. Oil analyses were performed at the USTA-CSIC laboratory using a gas chromatographer with a Flame Ionization Detector (Agilent 7820A, EZChrom Elite software). Samples were manually ground with liquid nitrogen; then, fatty acids were transformed into fatty acid methyl esters for detection following the procedure described by Lee et al. (2012) section 2.2.4 (supplementary Table S1). As a result, we obtained the absolute value of the oil content on a dry-weight basis and the percentage of each fatty acids type relative to the total oil content. The analysis identified 26 different fatty acids types in the studied species (see supplementary Table S2). This data set was used to conduct local-scale analysis of the variation in seed oil content and composition, together with seed mass data measured the 47 species, averaging the weight of five replicates of 50 dry seeds.

To contextualize our study at the regional level, and for comparison with non-alpine species, we retrieved seed oil and seed mass information for another 33 species native to Europe, congeneric with the species we collected from the Seed Information Database (SID) (2023) and classified as either generalist or strict lowland. In addition to our own seed oil data, we used these data to study regional altitudinal gradients, obtaining oil content and seed mass data for 80 species (29 strict alpines, 31 generalists and 20 strict lowlands) from 19 plant families.

### Seed germination and longevity

To estimate the earliness of germination, we used the EHS trait, calculated as the time (in days) to reach 50% germination, obtained from a previous phenology germination experiment conducted in the same system (Espinosa del Alba, Fernández-Pascual and Jiménez-Alfaro, 2024) and available for 36 of our study species. Longevity data was experimentally calculated for 35 species in the laboratory, using a standard comparative longevity protocol (Probert, Daws and Hay, 2009; Davies *et al.*, 2016) optimised for short-lived species (Davies *et al.*, 2016) and successfully applied to alpine species by Mondoni et al. (2011). The artificially accelerated ageing protocol allows the measurement of the p50 value (amount of time for seed viability to drop to 50%). The initial RH value was 30-35% (Hygropalm 3 display unit; Rotronic Instrument UK Ltd, Crawley, UK). Before the start of the ageing protocol, all species were tested to ensure germination above 85% (see specific germination conditions below) and species from the Cistaceae family were scarified with sandpaper to break physical dormancy. Seed samples (200 seeds/ species) were first rehydrated to 47% RH at 20ºC for five days in a non-saturated LiCl solution (Hay *et al.*, 2008) in crystal Petri dishes and kept in a 300 x 300 x 130 mm sealed electric enclosure box (Ensto UK Ltd, Southampton, UK) before moving the Petri dishes into the ageing conditions. The ageing conditions comprised a temperature of 45°C, 60% RH and darkness. Subsamples of 42 seeds were withdrawn after 2, 10, 15 and 30 days in ageing conditions and consequently sowed in Petri dishes 1% agar with GA3 following Kew standard procedure (Davies, Sacco and Newton, 2015). After the ageing period, those species requiring a cold stratification period (e.g., *Saxifraga oppositifolia*) were stored at 5ºC in darkness for a month before germination experiments. After ageing, germination conditions for the species were set with a 12/12 h photoperiod and two alternating temperatures: warmer conditions at 22-12ºC or 15/5ºC for those germinations which required colder temperatures (see supplementary Table S3). After sowing, seeds were checked once a week for four weeks. Germination was scored when there was a visible root (>1.5 mm) and then removed from the Petri dishes. After the end of the 28-day germination test, the ungerminated seeds were cut-tested under the binocular stereoscope to visually assess the embryo's state. We considered seeds with white and firm embryos viable, i.e. potentially germinable (Baskin and Baskin, 2014) and removed empty or infected seeds from further analysis.

We applied the viability equation

*v = Ki – (p/σ)*

where v is viability in NED (normal equivalent deviates), p is the days of ageing, Ki is the initial viability, and σ is the standard deviation of the distribution of deaths over time. Using GENSTAT software (REF), which applies this equation, we calculated p50, i.e. the time for viability to drop below 50%.

### Local ecological optimum

To calculate local species' ecological optimum, we established 20 additional plots (1 m²) per site (following the methodology of Jiménez-Alfaro et al., 2024) where we recorded all vascular species and buried a temperature datalogger at a depth of 5 cm (Thermochron, iButton, Newbury, UK; accuracy: ± 0.5 °C from -10 °C to +65 °C, resolution: 0.5 °C, records every four hours), which recorded temperatures over 11 months. From the microenvironmental data recorded, we calculated three bioclimatic indices per plot following Jiménez-Alfaro et al. (2024). (1) Growing Degree Days (GDD) as the sum of daily mean temperatures for days in which the soil mean temperature at five cm deep was above 5 ºC (Körner, 2021); (2) Freezing Degree Days (FDD) as the sum of daily mean temperatures for days in which the mean temperature was below 0 ºC (Choler, 2018); and (3) days with snow cover, based on the period in which the maximum temperatures were < 0.5 ºC and the minimum temperatures were > -0.5 ºC (Zhang et al., 2005). Then, we used the bioclimatic indices to calculate the species' local ecological optima, averaging the climatic variables of the plots where the species was present and weighting them by their coverage (only considering those plots where the species had more than 10% relative coverage), assuming species would have more coverage in those plots with climatic conditions closer to their local ecological optimum.

### Statistical analysis

We analyzed the seed trait data by fitting Markov Chain Monte Carlo generalized linear mixed models (MCMCglmm) with Bayesian estimation using the R package MCMCGLMM (Hadfield, 2010). For biological correlates, we investigated how seed oil and composition may explain other biological traits (seed mass, germination longevity), thus oil traits are used as the explanatory variable, both log-transformed. To meet normal distribution of the data, we applied a log transformation to seed mass and a square root transformation to p50 (EHS?). For the ecological drivers (altitudinal distribution and local ecological optimums i.e. GDD, FDD and Snow), we wanted to investigate how seed oil and composition are regulated by microclimatic gradients (across local snow-related factors), thus oil traits are used as the response variable, both log-transformed. We used weakly informative priors in all models, with parameter-expanded priors for the random effects. Each model was run for 1,000,000 iterations, with an initial burn of 100,000 and a thinning interval of 100. We calculated mean parameter estimates and 95% credible intervals (CI) from the resulting posterior distributions. Phylogeny was included as a random factor using a reconstructed tree (Supporting information Figure S1 and Figure S2 for regional and local species pool, respectively), created with V.PHYLOMAKER R package (Jin and Qian, 2019) using phylosignal and phylobase R packages (Keck *et al.*, 2016; R Hackathon et al., 2020). Add phylogenetic signal calculations.

## 3.Results (1066 words)

### Seed oil content and oil composition

Within our local species pool (n=47), oil content varies from 1.2% in *Avenella flexuosa* (Poaceae) to 34.2% in *Jasione cavanillesii* (Campanulaceae) (Figure 2A). The most abundant fatty acids in alpine seeds were the unsaturated linoleic acid (C18:2n-6c, 42.4%±2.4, mean ± se), oleic acid (C18:1n-9c, 22.6%±1.9), and alpha-linolenic acid (C18:3n3, 15.1%±2.7); as well as saturated palmitic (C16:0, 10.8%±0.8) (details in supplementary Table S2). These four averages 94.8% of seed oil content (se=23). Additionally, in our data set, erucic acid (C22:1n9) had high values only in Brassicaceae and gamma-linolenic acid (C18:3n6) in some Caryophyllaceae (Figure 2B). The mean frequency of saturated fatty acids is 14.2%±0.95, and the mean ratio between unsaturated and saturated fatty acids is 7.2 (se=0.5), ranging from 1.3 in *Avenella flexuosa* up to 20.1 in *Iberis carnosa* (Brassicaceae). The families Poaceae, Salicaceae, and Cistaceae generally had low UFA/SFA ratio values, indicating higher saturated fatty acids synthesis. At the same time, Brassicaceae and Lamiaceae were characterized by higher ratio values, showing a tendency to synthesize more unsaturated fatty acids (Figure 2C).

We conducted a Principal Component Analysis for plant families using fatty acid values with >3% relative proportion and correlations below 0.7 to reduce dimensionality (Figures 3D and 3E). The UFA/SFA ratio contributed the most to PC1 (26.4%) followed by C16:0 (18.6%), while C20:1n9 and C22:1n9 mainly contributed to PC2 (21.9% and 16.3%, respectively). Additionally, PCA revealed that the oil content percentage is not highly correlated with any specific fatty acids type (details in supplementary Table S4).

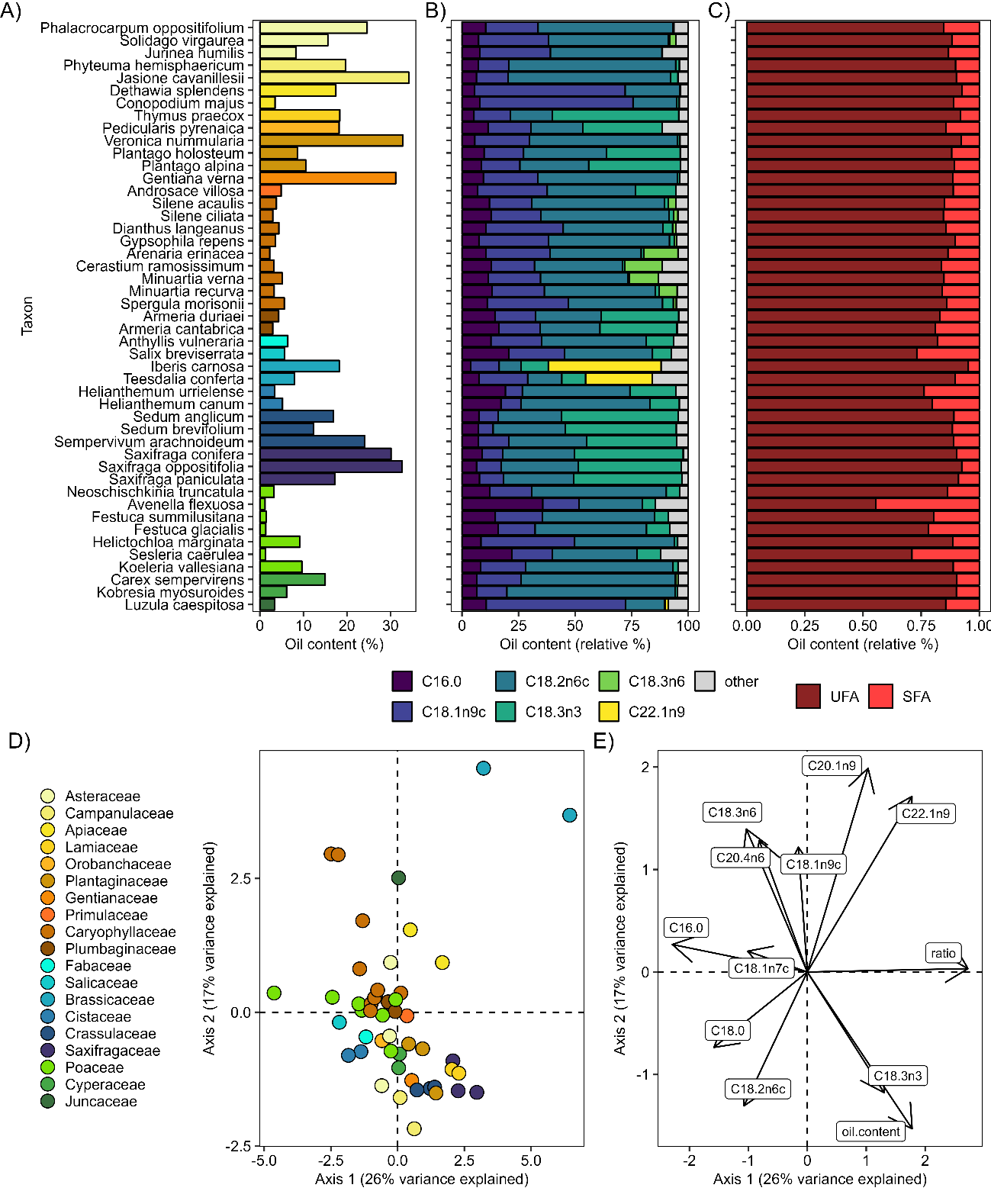


Figure 2. Local species oil content and composition data exploration (n=47). A) Oil content (%) per species, colours representing families as panel D. B) Seed oil composition of fatty acids with more than 10% relative proportion on average across species. C) Seed oil composition per species with fatty acids divided between Unsaturated Fatty Acids (UFA) and Saturated Fatty Acids (SFA). D) Exploratory PCA with species as points and E) variables directions and contributions.

In our regional dataset with 80 species analyzed (Figure 3A), we found no significant oil content differences between strict alpine, generalist and strict lowland species (model details in supplementary Table S5). Similarly to the patterns found at local scale, oil content values ranged from 1.19% to 38%, with a mean value of 11.5%. Additionally, we found no significant correlation between oil content (%) and seed mass (mg) (posterior mean=-0.064). We observed a considerable variation of seed oil content in smaller seeds, while variation was reduced with increasing seed mass (Figure 3B). Seed mass values ranged from 0.64 mg to 335 mg, with a mean value of 51 mg.

A comparison of a graph

Description automatically generated with medium confidence

Figure 3. Regional oil content patterns (n=80 species). A) Seed oil content (%) between regional altitudinal distribution showed by different colours. B) Seed oil content (%) and seed mass (mg) non-significant relationship (MCMC-GLMMs), colours by families.

### Biological correlates

Seed mass values ranged from 0.6 mg (*Sedum brevifolium*) to 268 mg (*Jurinea humilis*), with a mean of 45.4 mg (se=7.84). Although we observed a general trend of decreasing seed mass with higher proportion of seed oil content, the relationship was not significant (posterior mean=-0.15, Figure 4A, left panel). Seed Oil composition calculated as the UFA/SFA ratio had no visible effects on seed mass and the relationship was also not significant (posterior mean=-0.03, Figure 4A right panel). The p50 values ranged from 3 to 47 days, with a mean of 21 days (se=2). Higher oil content significantly reduced seed longevity (posterior mean=-0.83, Figure 4B left panel). The relationship for the ratio was only marginally significant; seeds with a higher UFA/SFA ratio (i.e. more unsaturated fatty acids) showed a decrease in longevity (posterior mean=-0.84, Figure 4B right panel). Earliness of germination, estimated as EHS, ranged from 4 to 295 days, with a mean of 150 days (se=16.6). We did not find any significant relationship between EHS and oil content (posterior mean=31.7, Figure 4C left panel) or the UFA/SFA ratio (posterior mean=79, Figure 4C right panel). Despite the non-significance, we observed a trend of seeds with higher oil content and higher UFA/SFA ratio experiencing delayed germination.

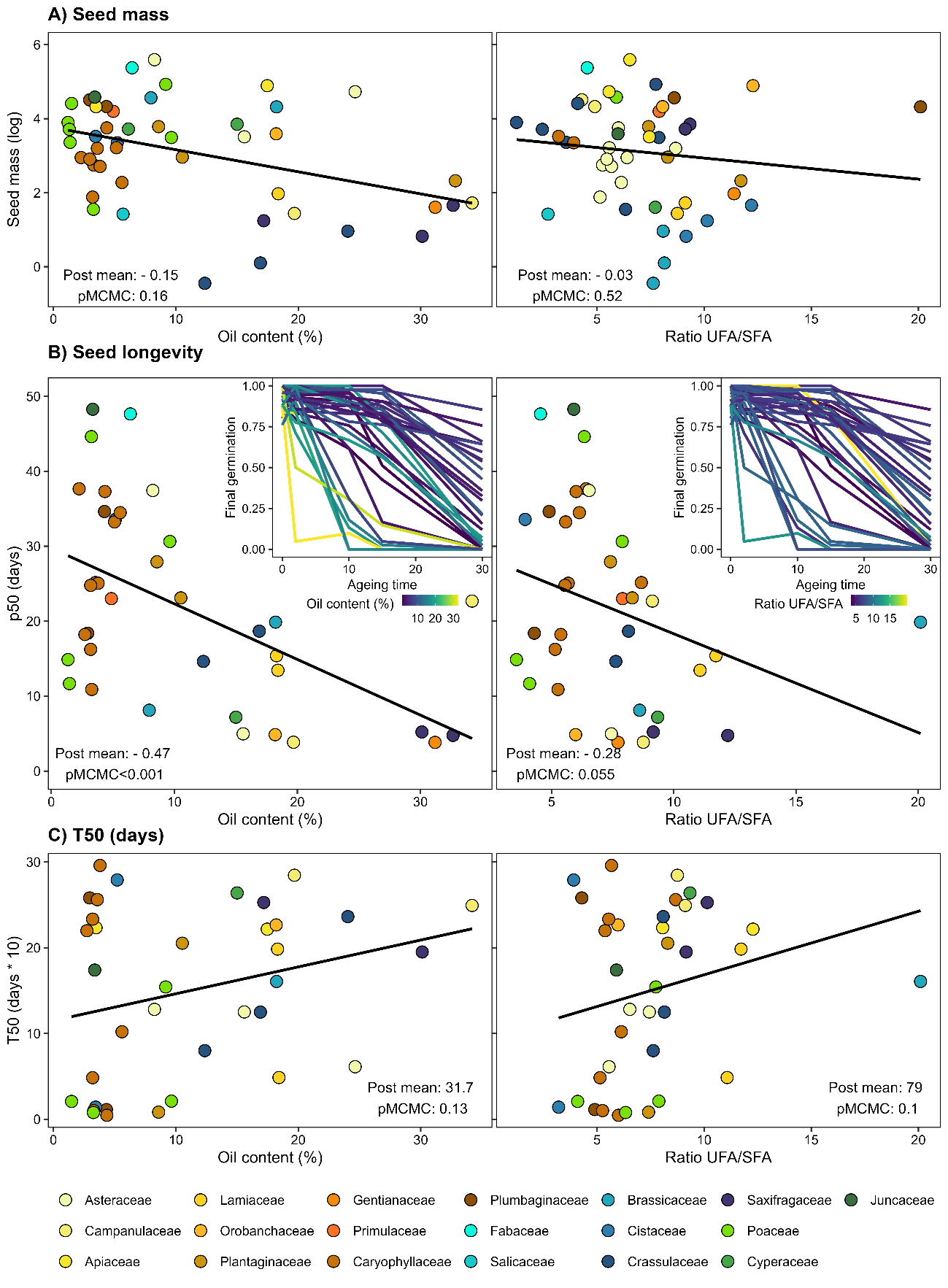


Figure 4. Seed oil content and ratio UFA/SFA biological trade-offs. A) Correlation between seed mass, oil content, and ratio UFA/SFA, significances from MCMC-GLMM models (Gaussian family, n=47) with all variables log-transformed. B) P50: time for viability to drop to 50%, based on probit analysis (GENSTAT software). C) Germination timing, EHS: depending on oil content and ratio UFA/SFA. Significances from MCMC-GLMM (n=36), both explanatory variables log-transformed. Colours represent the different families.

### Ecological drivers

GDD values ranged from 650 to 2295 °C, averaging 1421 °C (se = 67.9), while FDD values ranged from 0.05 to 170 °C, with a mean of 31.8 °C (se = 4.7). We found a general trend of species with lower oil content and a lower UFA/SFA ratio placed in warmer conditions (high GDD and low FDD) along local microclimatic gradients (Figure 5), although these effects were no significant. The trends were more consistent for GDD, since the effect of FDD was mainly driven by a few species with more extreme oil content and composition values. In addition, snow values ranged from 3 to 157 days, with an average of 62 days with snow per year. The data showed a trend where species with a preference for snowier sites showed higher oil content and UFA/SFA ratios, but with no significant effects. Details of the model results are shown in supplementary Table S4.

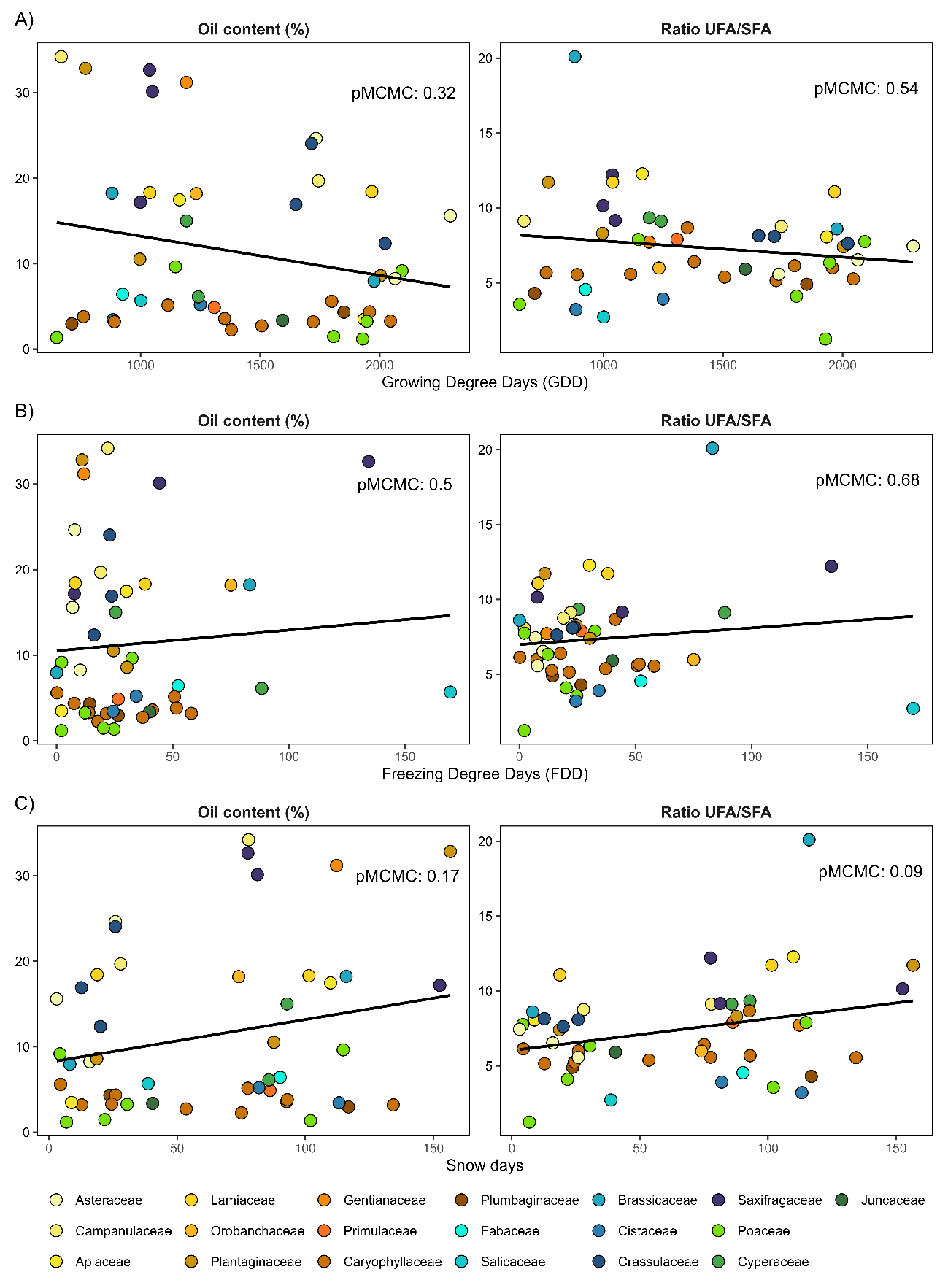


Figure 5. Seed oil content ecological trade-offs, oil content and ratio UFA/SFA log-transformed for MCMC-GLMM models (family=Gaussian, n=47). A) Relationship with GDD (Growing Degree Days °C); B) Relationship with FDD (Freezing Degree Days °C) and C) Relationship with Snow days.

## 4. Discussion (1269 words)

This study aimed to explore biological correlates and ecological drivers of seed oil content and seed oil composition in a regional pool of 47 alpine species. We found strong indications that, in alpine species, seed oil content and composition significantly influence seed longevity, potentially impacting soil seed banks and conservation strategies for seed bank managers or restoration programmes. Interestingly, we found little evidence of temperature-related patterns pointing to macroevolutionary processes driving seed oil content and composition at the regional or local scales.

We found low correlations (< 0.7) between total oil content and other fatty acids, in contrast to Sanyal and Decocq (2016) who found several significant negative relationships between specific fatty acids. While oil content is known to vary globally from 1% in *Musa paradisiaca* to 76% in *Chrysobalanus icaco* (Matthäus, 2012), our alpine species show a reduced but still relevant range of seed oil percentages (1-38%). This variation is similar to the results found in other studies focused on herbaceous plants, like the one exhibited by weed species in France (Bretagnolle et al., 2016). These patterns are in line with the general variation in seed oil predicted for herbaceous and woody species in the pioneer studies of Levin (1974). The seed oil composition we found in our alpine species was also comparable to what Bretagnolle et al. (2016) reported for weed species, except for erucic acid (C22:1n9) in Brassicaceae. Previous reports from other commercial Brassicaceae species confirmed high proportions of elongated acyl chains from C20 to C24 in this family (Baud and Lepiniec, 2010). The high levels of erucic acid are surprising due to its “high” melting point (33.5°C) (Sanyal and Linder, 2013), which would hinder energy release; however, erucic acid has also been associated with lower seed predation (Jong et al., 2016).

Within our alpine system, we could not corroborate the expected negative relationships between seed mass and seed oil content described in the literature (Nagel and Börner, 2010; Hamilton et al., 2013; Bretagnolle et al., 2016). A plausible explanation for this result is that most studies reporting significant relationships include different vegetation types and multiple comparisons among herbs, shrubs, and trees. In contrast, our study focused on similar life forms, mostly herbaceous hemicryptophytes and dwarf chamaephytes, where seed mass and other biological traits are expected to be more constant (Diaz et al. 2016). Similar non-significant trends were reported in another study focused on trees (Finkelstein and Grubb, 2002), suggesting that global variation in seed variation is constrained by life forms. Nevertheless, more studies across different life forms and vegetation types are still necessary to confirm this idea. In our local alpine species pool, we also found a negative trend between seed mass and UFA/SFA ratio. However, this trend contradicts the expectation of small seeds storing more saturated fatty acids, i.e. lower ratio values (Linder, 2000). We found a considerable variation of oil content in small seeds, although such variation strongly decreases towards low oil values in large seeds, supporting the results reported by Bretagnolle *et al.* (2016). Nevertheless, there appears to be a strong phylogenetic trend (Levin, 1974), with species from the same family mostly grouped together.

In concordance with our expectations, we found a significant negative relationship between seed longevity and seed oil content and composition. Alpine seeds, which are generally known for their short longevity (Mondoni et al., 2011), showed a consistent decrease in seed longevity with increasing oil content and UFA/SFA ratio, indicating more unsaturated fatty acids. This finding supports the hypothesis that oily seeds have lower longevity (Nagel and Börner, 2010; Neto et al., 2019) and that a higher proportion of unsaturated fatty acids is associated with shorter longevity (Hoekstra, 2005). Our results are consistent with previously described adverse effects observed in seed longevity, possibly due to lipid oxidation (Bailly et al., 1998; Tammela et al., 2003) and contrast with other studies reporting no relationship between seed longevity and oil content (Walters, Wheeler and Grotenhuis, 2005; Probert, Daws and Hay, 2009; Gardarin et al., 2010). With the current biodiversity crisis, plant conservation is a global priority, especially ex-situ conservation in seed banks. Having the necessary knowledge and appropriate methods to store and maintain seed viability in these facilities is vital. The results presented here suggest that, for alpine species, seed oil content can be an important factor to consider. Measuring seed oil content and composition is a destructive yet fast method that can be used to separate those alpine species with particularly low seed longevity.

Based on previous studies by Sanyal and Decocq (2016) and Linder (2000), we predicted that alpine species, adapted to live and germinate under colder temperatures, would show earlier germination with increasing oil content and an increasing UFA/SFA ratio. Surprisingly, the negative relationship between oil content and the earliness of germination contradicted our initial expectations. The lack of patterns with oil content is not completely surprising in wild species, as has been reported in rainforest species in Australia (Hamilton et al., 2013), whereas weeds (Gardarin, Dürr, and Colbach, 2011) and crops (Gu et al., 2019; Cheng et al., 2024) have shown positive correlations. Observing this pattern between wild and cultivated species, one could say that crop selection has effectively favoured varieties exhibiting both high oil content and early germination; however, there is an important data gap of seed oil content in wild species. We also observed the opposite expected trend with species with higher unsaturated fatty acids germinating later against Linder (2000). A plausible explanation could be that alpine species' physiological seed dormancy constraints (Schwienbacher et al., 2011) drive germination patterns more strongly than oil content and composition.

Against our expectations, we found no significant differences along the altitudinal gradient at the regional scale and did not find any significant relationship with the species' local ecological optima. Previous research in a few cultivars also failed to detect a significant positive correlation between seed oil and elevation (Dierig *et al.*, 2006; Cai *et al.*, 2012). These results might indicate a low effect of elevation in seed oil content (Dierig *et al.*, 2006), although there are reports of a positive correlation between oil content and mean temperatures (Piper and Boote, 1999). Parallelly, Cai *et al.* (2012) did find a positive correlation between elevation and percentage of unsaturated fatty acids within a single species grown in a broad altitudinal range. None of the local microenvironmental gradients studied (GDD, FDD and snow) showed any significant relationship with oil content or composition. However, snow days demonstrated a more pronounced relationship with the UFA/SFA ratio, indicating that species living in more snowy sites tended to have higher unsaturated fatty acids, which could potentially be consistent with the anti-freezing properties that unsaturated fatty acids can provide (Linder, 2000). The lack of regional and local patterns seems to limit Sanyal and Linder's claims (Sanyal and Linder, 2013; Sanyal and Decocq, 2016) of strong temperature selection at macroevolutionary level constraining seed oil patterns. These results might indicate a strong phylogenetic signal for oil content. However, providing a more complete picture is difficult due to the limited information on oil content and fatty acid composition available for wild species (Levin, 1974).

Although the patterns presented in the study provide new insights into the understudied topic of seed oil and composition and their trade-offs in wild species, we must assume some caveats and limitations in our study. For the complete seed oil and seed traits dataset, analyses and measurements were done in two or three batches, corresponding with the seed availability after each year's collection campaign. Nevertheless, we are confident that we have collected fully mature seeds, following the ENSCONET protocol (2009), and have applied a consistent methodology for all experiments and analyses.

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