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

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Abbreviations:

FA: Fatty Acids; UFAs: Unsaturated Fatty Acids; SFAs: Saturated Fatty Acids

## Summary/abstract

We will use a combination of techniques laboratory-based and physiological and ecological seed traits. Seed traits like oil content could be a potential predictors for many biological responses.

## Introduction

### Seeds reservoirs: oil content

Seeds act as reservoirs of energy accumulating three main macromolecules: proteins, lipids and carbohydrates (Levin, 1974; Baud and Lepiniec, 2010). The quality of these reserves is directly related to plant fitness and it influences a wide range of biological processes (REF). 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 (FA) (Voelker and Kinney, 2001), and the latter accumulated as starch (Bretagnolle *et al.*, 2016). Carbons in FAs are highly reduced and, through beta-oxidation, they release more than twice as much energy as the oxidation of starch or proteins on a per g basis of dry weight (Levin, 1974; Baud and Lepiniec, 2010). Consequently, plants mostly rely on lipids (hereafter “seed oil” for simplicity) stored in the seed for energy in the first life stages (REF). Seed oil is mainly stored in the cotyledons and endosperm (Ellis, 2006) but also in the radicle and hypocotyl (Li *et al.*, 2006). Although oil content variation is found within and among genera of the same family (Levin, 1974; Bretagnolle *et al.*, 2016) it is also highly constrained by phylogeny and subject to evolutionary change (Levin, 1974).

### Seed oil composition

In seeds, most FAs range from 10 to 22 carbons in length and the carbons may be joined by single or double bonds (REF). FAs with one or more double bonds are referred to as unsaturated (UFAs) and FAs without double bonds are referred to as saturated (SFAs) (Ellis, 2006). The relative proportion of specific FAs as well as the relative proportion of UFA and SFA is what we call “oil composition”. The most abundant FAs found in seeds are the unsaturated oleic acid (18:1n-9, OLA), linoleic acid (18:2n-6, LA) and a-linolenic acid (18:3n3, ALA) and the saturated palmitic (16:0, PA) and stearic (18:0, SA) acids (Voelker and Kinney, 2001; Ellis, 2006; Baud and Lepiniec, 2010). SFAs and UFAs have differential biochemical properties 41 that influence the responses of seeds to biological processes and to the environment. On a per-carbon basis, UFAs cost more to produce and yield less energy when oxidized than SFAs 41 (Linder, 2000). Moreover, the storage of UFAs needs the storage of antioxidant molecules to prevent damage due to the higher potential for oxidative damage (Benson 1990). Hence, a maximal storage strategy for seeds should maximise SFA storage instead of UFA (Linder, 2000). However, the relative abundance of UFAs and SFAs highly varies in angiosperms (Voelker and Kinney, 2001) and, contrary to the expectation, many species synthesise a very low amount of SFAs (Linder, 2000), a potential explanation found in the literature is that UFAs are more unpalatable and also have anti-freezing properties (Linder, 2000). Another important point is that SFAs have higher melting points than UFAs, (Benson 1990) (Sanyal and Decocq, 2016) which influences the availability of these energy resources.

### Importance

Oil content and composition modify seed responses (Hoekstra, 2005) influencing seed dormancy and viability, germination potential (Westoby et al., 1992) and success (Linder, 2000) as well as the emergence and establishment of a plant [Bewley 1994]. Consequently, seed oil content and composition are crucial for plant adaptation (Sanyal and Decocq, 2016) and validate the adaptive hypothesis (Linder, 2000), also suggesting that selection could be acting simultaneously on multiple seed traits, like seed mass (Sanyal and Decocq, 2016). Both oil content and composition vary in response to environmental, geographical location and maternal genotypes (Linder, 2000; Ghebretinsae *et al.*, 2008). Seed oil content and oil composition seem to be under strong selection by temperature at both micro- and macro-evolutionary levels (Sanyal and Linder, 2013; Sanyal and Decocq, 2016). Despite their importance, very few studies on native species address and analyse seed oil content and composition compared to the abundant specific studies in commercial species (cotton, brassica, soybean) especially focused on plant breeding programs to increase oil content that could be used for biofuel (ref).

### Seed mass and oil content

Seed mass is one of the most studied seed traits and oil weight can contribute up to 60% of seed mass (Ellis, 2006). There is a consensus in the literature that seed mass and oil content are negatively correlated while it is also known that stored seed energy and oil content are positively correlated (Levin, 1974; Bretagnolle *et al.*, 2016). Thus, large seeds generally store less oil than small seeds, whereas small seeds have higher oil content. Nevertheless, a small oily seed can release as much energy as a starchy seed that is twice as heavy. Such a relationship suggests that as oil synthesis is energetically costly relative to carbohydrates, oil synthesis could be an advantage only for small seeds which can store more energy in a smaller volume (Bretagnolle *et al.*, 2016), and should maximise SFA storage instead of UFA (Linder, 2000).

### Seed longevity and oil content.

Seed longevity, i.e. the ability of seeds to remain viable over certain storage periods, is determined by an intricate network of genetic factors, taxonomic differences (Walters, Wheeler and Grotenhuis, 2005) and environmental factors. Species from cold and temperate climates have shorter longevity than seeds of species from hot and arid climates (McDonald, 1999; Kranner et al., 2010; Walters et al., 2010 (Kochanek 2010(Probert, Daws and Hay, 2009) Mondoni 2012?. Oil content influences seed viability (Hoekstra, 2005) and longevity (Bewley et al., 2013) because during lipid oxidation it generates free radicals and reactive oxygen species (ROS); Hendry, 1993; Bailly, 2004), which can cause detrimental effects on membrane integrity (Priestley and Leopold, 1979) (Kranner et al., 2002) and lead to mitochondrial dysfunction, enzyme inactivation, membrane perturbation and genetic damage (Coolbear, 1995). Particularly, the oxidation of UFAs is highly contributing to free radicals’ production and subsequent attacks on other macromolecules (Benson 1990). To prevent the deleterious effect of lipid oxidation, oily seeds also store antioxidants, and supporting findings show a positive correlation between antioxidant levels and the relative proportion of UFAs (Kamal-Eldin & Andersson, 1997; Sattler et al., 2004; Falk & Munn\_e-Bosch, 2010). Thus, not only oil content but also oil composition, i.e. the relative proportions of UFAs and SFAs affect the storage behaviour of seeds (Walters et al. 2004; Volk et al. 2006; Walters et al. 2015).

Accordingly, previous studies have found oily seeds to be more sensitive to ageing (Nagel and Börner, 2010; Neto *et al.*, 2019) and detailed studies including oil composition have also shown that a higher proportion of UFAs are associated with shorter longevity (Ponquett et al 1992) (Hoekstra, 2005). However, in general, the correlation between seed oil content and longevity has been described as weak (Nagel and Börner, 2010) Priestley et al., 1985; Walters et al., 2005) and even no significant relationships have been reported (Probert, Daws and Hay, 2009; Gardarin *et al.*, 2010) (Mederios et al 1998). Further investigations on the effects of seed oils (content and composition) on longevity are desirable (Pritchard and Dickie, 2003) but lacking for the vast majority of wild species.

### Global seed oil content patterns

Few studies have tried to describe global seed oil content patterns. In 1974, (Levin, 1974) found that significant seed oil content variations depending on habitat type, (oil increased with woodiness and shade tolerance), and life form (lower oil in herbs than in shrubs than in trees) but no geographical differences between temperate, subtropical and tropical regions. However, a more recent study by (Sanyal and Decocq, 2016) found that seed oil content to be significantly higher in tropical plants compared to temperate plants, probably because seeds need higher energy to survive first life stages with high competition and/or low illumination (Levin, 1974) (Salisbury 1942). In the same study also found higher proportions of UFAs (oleic and eicosenoic) with increasing latitude (Sanyal and Decocq, 2016) in concordance with previous studies (Linder, 2000; Sanyal and Linder, 2013), with the relative proportions of SFAs decreasing by 0.1% for each degree of latitude (Sanyal and Linder, 2013). Additionally, due to UFAs and SFAs different melting points, at lower latitudes and altitudes with higher temperatures, seeds with higher proportions of SFA would be favoured because they would have more energy for growth without delaying or slowing germination (Sanyal and Decocq, 2016). At higher latitudes/altitudes and thus cooler temperatures, seeds that have a higher proportion of UFAs may germinate earlier and/or more (Linder, 2000). Rich UFAs seeds could then germinate faster and earlier than rich SFAs seeds in cold conditions, providing a competitive advantage where cold temperature regulates seed germination (Linder, 2000).

### The alpine environment

To our knowledge, seed oil content and composition have never been researched in alpine species although is known to influence a wide range of biological processes and ecological correlates (other REFS from intro). The alpine environment is characterised by harsh environmental conditions and strong microclimatic gradients even at short distances (Scherrer and Körner, 2011) that physiologically limit plant regeneration (Körner, 2021). Alpine species have evolved under these circumstances and thus adapted, including the oil content and composition, to maximise the chances for successful regeneration. Alpine species are generally known for having small seeds (REF), delayed germination phenology (Fernández-Pascual et al 2021, Espinosa del Alba et al 2024) and short longevity (Mondoni 2011) which we expect to correlate with oil content and composition. We also expect to find micro and macroscale oil patterns related to temperature according to the results reported by Sanyal et al 2013 and 2016.

### Aims, questions and predictions.

The goal of the study is to explore the patterns of seed oil content and composition in alpine plants and understand their biological and ecological correlates. This kind of data is barely available for alpine species. Our study is structured in 3 main sections/questions:

1. Regional scale. Explore and describe seed oil content in alpine species at regional scales. Question: Does oil content follow global seed oil content patterns?
   1. H1.1. Strict alpine species (specialists) will have less oil content than generalist species and strict lowland. Due to the lower temperatures in alpine. (NS differences with 82 species data from literature).
   2. H1.2. Negative correlation between seed mass and oil content (NS).
2. Local scale. Explore seed oil content/composition biological correlates with seed mass, seed longevity (p50) and germination timing (t50, time to reach 50% of germination). Question: How do oil content and oil composition (ratio UFA/SFA) correlate with seed mass, seed longevity (p50) and germination timing t50 (time to reach 50% germination)?
   1. H2.1. Higher seed mass will correlate with less oil content (trend but NS) and more UFA/SFA ratio (NS).
   2. H2.2. Less longevity will correlate with higher oil content (corroborated \*) and a higher UFA/SFA ratio (marginal). Oil generates ROS (especially UFAs) and thus decreases longevity.
   3. H2.3. Faster germination (lower t50) will correlate with higher oil content (NS) and higher UFA/SFA ratio (NS).
3. Local scale. Ecological correlates at local scale. Does oil content and composition (ratio UFA/SFA) correlate with the local ecological optimal of the species/community (GDD, FDD, Snow)?

## Methods

### Study system

We focused on alpine species from grassland communities in the Cantabrian mountains (North-western Spain) a transitional mountain hub between Eurosiberian and Mediterranean regions in Europe (Jiménez-Alfaro *et al.*, 2021). 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. Grassland communities are mostly dominated by *Poaceae* and *Cyperaceae* and the main lifeforms are Hemicryptophytes and Chamaephytes. Local richness ranges from 4 to 28 species and grazing impact is restricted to wild populations of Cantabrian chamois (*Rupricapra pyrenaica parva*). Climatic conditions follow a north-south temperature and precipitations gradient with colder and wetter conditions in northern slopes compared to warmer and drier conditions in southern slopes.

### Species data

We established eight sampling sites, four in northern slopes and four in the southern slopes of the Cantabrian Mountains to ensure representation of the flora from the two biogeographical regions. In each sampling site, we collected floristic and community composition data for 119 alpine grassland species. We collected enough seeds from 47 species of those species (200mg of dry seeds), the data obtained about FAs content and composition of these species were not known before.

The collected species were classified between strict alpine and generalist according to their ecology. We identified plant specialists as those significantly associated with the target vegetation type (alpine grasslands), using the Indicator Values (IndVal) in the indicspecies R package (REF). The calculations were based on 12,000 vegetation plots of grasslands stored in the SIVIM database for the Cantabrian Mixed Forests ecoregion (paper classification). From the preliminary list of indicator species for the studied vegetation, we removed species with median elevation values below 1800 m, most of them characteristic of subalpine or nitrophilous habitats and those were specified as generalist.

### Oil content and composition.

We preliminary explored within-accession seed oil content variation in five species analysing three subsamples from each of those (also to ensure high precision of methodology), and we could not detect any statistical differences between subsamples. Our preliminary results were also consistent with the analysis of 360 accessions of Arabidopsis thaliana which contained identical fatty acids with slight variation in the relative proportion [9,10]. Consequently, a single sample for each species seed accession was analysed, thus within-species seed oil variation was not explored. We sent the samples to an external analytical laboratory (USTA-CSIC) where they used 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 to fatty acid methyl esters (FAMEs) following the procedure described by Lee et al., (2012 add ref) section 2.2.4 (supplementary table xx in appendix with complete list of Fame’s possible to identify). As a result, we obtained the oil content on a dry-weight bases and the percentage of each FAME’s type relative to the total of oil content. The analysis identified 26 different FAME types in the studied species (see supplementary table xx). Additionally, we retrieve oil and seed mass information of another 33 species, classified as either generalist or strict lowland, congeneric of the species present we analysed, from SID database (REF). Our oil data in addition to the data obtained from SID database was used for regional scale analysis, containing oil content and seed mass data for 80 species (29 strict alpine, 31 generalists and 20 strict lowland) from 19 different plant families. For local scale analysis, we used only our own oil data, containing seed oil content and oil composition data for 47 species from 19 families.

### Seed trait data

Seed mass data was calculated for all species averaging the weight of five replicates of 50 dry seeds. To estimate germination timing we used T50 trait, calculated as the time (in days) to reach 50% germination, obtained from a published phenology germination experiment, done with species from the same study area (Espinosa del Alba et al. 2024) and is available for 36 species. Under snow germ trait 44/50??.

Longevity data was experimentally calculated for 31 species. We applied 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 artificial accelerated ageing protocol allows the measurement of p50 value (amount of time for seed viability to drip 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% and for those species from the Cistaceae family were physically scarified with sandpaper. Seed samples (200 seeds per 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 in a 300 x 300 x 130 mm sealed electric enclosure box (Ensto UK Ltd, Southampton, UK) before moving the vials into the ageing conditions. The ageing conditions consisted of a temperature of 45°C, a RH of 60% and darkness. A subsample of 42 seeds was withdrawn after 2, 10, 15 and 30 days in ageing conditions and consequently sowed in petri dishes 1% agar with 250 ml/L of GA3 (Kew Royal Botanic Garden Technical Information sheet\_13a). For those species which required a cold stratification period (*Saxifraga oppositifolia*), after ageing period, were put in a refrigerator at 5ºC and darkness for a month before starting germination experiments (same procedure followed by Satyanti *et al.*, 2018). 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 table xx for details). After the sowing, the 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 state of the embryo. We considered seeds with white and firm embryos viable, i.e. potentially germinable (Baskin and Baskin, 2014) and we removed empty or infected seeds from further analysis.

We applied the viability equations developed by seed bank managers

*v = Ki – (p/σ)*

where v is viability in NED (Normal equivalent deviates), p are the days of ageing, Ki is the initial viability, σ is the standard deviation of the distribution of deaths over time. Using GENSTAT software (REF), which applies this equation, we calculated the P50, i.e. the time for viability to drop below 50%.

### Species ecological preferences

To calculate species' ecological preferences we measured the microenvironmental gradients establishing 20 additional plots (1m2) for sampling site, five in each cardinal direction with a 10 m separation where we registered the relative abundances of all vascular species (following the methodology (Jiménez-Alfaro *et al.*, 2024). In total, we established 160 vegetation plots (8 sites x 20 plots) where we also buried an iButton datalogger, buried at 5 cm deep, in each vegetation plot (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 across 11 months.

From the environmental data recorded, we calculated a 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 temperature were > -0.5 ºC (Zhang 2005 ). Then we use the bioclimatic indices to calculate species' ecological preferences averaging the climatic variables of the plots where the species was present and weighted by its coverage (only considering those plots where the species had more than 10% of relative coverage). Assuming that species would have more coverage in those plots with climatic conditions closer to their preferences.

### Statistical analysis

We use GENSTAT software to apply a Probit analysis (Hay, Mead and Bloomberg, 2014) and calculate p50 values.

We analyzed the data by fitting Markov Chain Monte Carlo generalized linear mixed models (MCMCglmm) with Bayesian estimation using the R package MCMCGLMM (Hadfield, 2010). To model raw germination scores from longevity experiment, we used binomial MCMCglmms (family = multinomial2) while for the rest of traits we scaled the values and used gaussian MCMCglmms (family = gaussian). Our two response variables are total oil content (in percentage) and UFA/SFA ratio (both log-transformed) and the explanatory variables were seed mass, P50 and T50 for biological correlates; and their ecology (strict alpine, generalist or strict lowland) as well as their local ecological optimums. When needed, traits were log transformed to ensure the normality of the data distributions. In all models, we used weakly informative priors, 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. From the resulting posterior distributions, we calculated mean parameter estimates and 95% credible intervals (CI). Phylogeny was included using a reconstructed tree (Supporting information Fig. **S1**), 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).

## Results

### Regional oil content patterns

We found no significant oil content (%) differences between strict alpine, generalist and strict lowland species (Figure 2A) from our regional dataset (N= 80). 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). We observed a large variation of seed oil content in smaller seeds while variation was reduced with increasing seed mass (Figure 2B). Seed mass values ranged from 0.64 mg to 335 mg with a mean value of 51 mg.

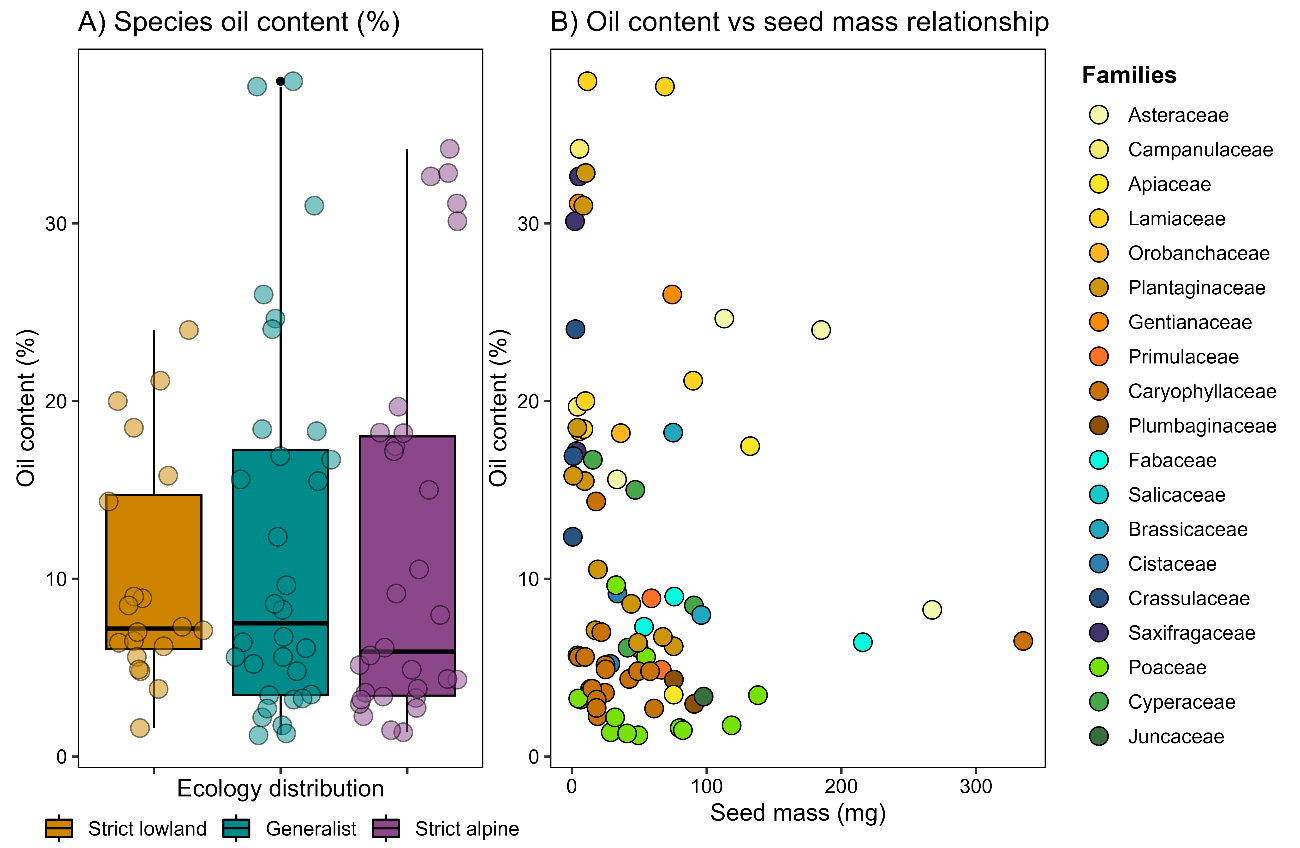


Figure 2. Regional oil content patterns (N = 80 species). A) Seed oil content (%) between species regional distribution showed by different colours. B) Seed oil content (%) and seed mass (mg) non-significant relationship (MCMC-GLMMs), colours by families.

Congeneric species has very similar levels and proportions of FA and oil content (as stated in literature, and see figure XX in supplementary?)

### Local oil content and oil composition patterns

Within our species, oil content varies from 1.2% in *Avenella fleuosa* (Poaceae) to 34.2% in *Jasione cavanillesii* (Campanulacea) (Figure 3A). Most abundant FAMEs in alpine seeds were the unsaturated linoleic acid (C18:2n-6c, LA, 42.4%±2.4, mean ± se), oleic acid (C18:1n-9c, OLA, 22.6%±1.9) and alpha-linolenic acid (C18:3n3, ALA, 15.1%±2.7); and saturated palmitic (C16:0, PA, 10.8%±0.8) (Detailed values for all FAME’s types in supplementary table XX). These four represent a mean of 94.8% of seed oil content (se = 23). Additionally, in our data set, Erucic acid (C22:1n9) had also high values only in Brassicaceae species and gamma-linolenic acid (C18:3n6) in some Caryophillaceae species (Figure 3B). The mean frequency of saturated fatty acids (SFA is 14.2%±0.95) and the mean ratio between unsaturated fatty acids (UFA) and SFA is 7.2 (se= 0.5), ranging from 1.3 in *A. flexuosa* up to 20.1 in *Iberis carnosa* (Brassicaceae). In general, the Poaceae, Salicaceae and Cistaceae had low values of the UFA/SFA ratio, indicating higher synthesis SFAs, while other families such as the Brassicaceae and Lamiaceae were characterised by higher ratio values showing a tendency to synthesise more UFAs (Figure 3C).

We restricted the exploratory PCA to those FAME’s with >3% relative proportion and with correlations below 0.7 to reduce dimensionality. The multivariate analysis showed relatively low explained variation within the first two axes (26.1% and 16.7% respectively). UFA/SFA ratio and C16:0 FAMEs were contributing most in the PC1 (26.4% and 18.6% respectively) and of C20:1n9 and C22:1n9 are the ones contributing the most PC2 (21.9% and 16.3% respectively) (Figures 3D and 3E). Additionally, PCA revealed that oil content percentage is not highly correlated with any specific FAMEs type (table in supplementary).

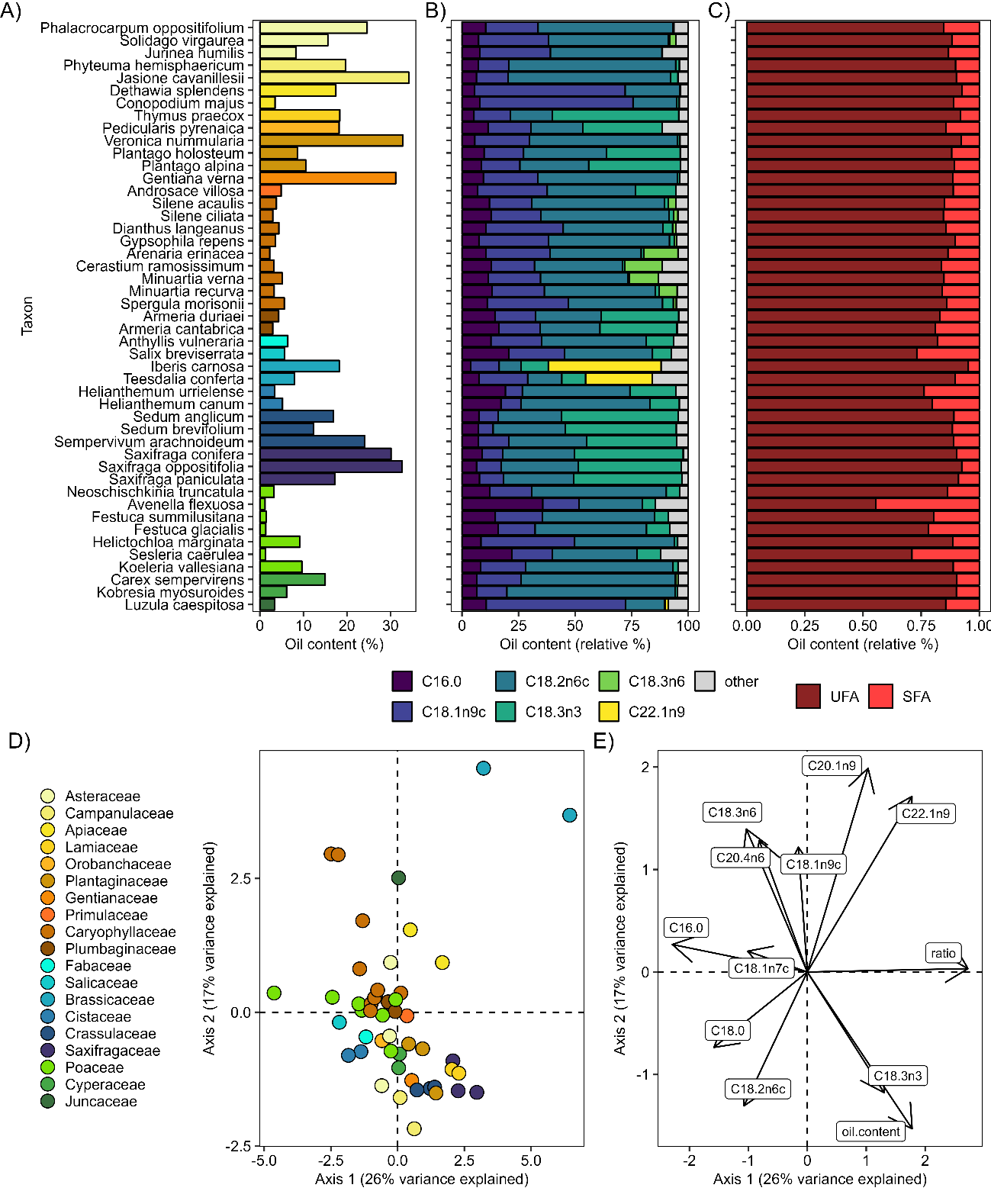


Figure 3. Local oil content and oil composition data exploration (N=47 species). A) Oil content (%) per species in percentage, colours representing families as panel D. B) Seed oil composition of FAME’s with more than 10% of relative abundance in average across species. C) Seed oil composition per species with FAME’s divided between Unsaturated Fatty Acids (UFA) and Saturated Fatty Acids (SFA). D) Exploratory PCA with species as points and E) variables directions and contributions.

### Biological correlates

#### Seed mass

Seed mass values (dry weight 50 seeds) ranged from 0.6 mg (*Sedum brevifolium*) to 268 mg (*Jurinea humilis*), with a mean of 45.4 mg (se=7.84). We observed a notable variation of oil content in small seeds, but its variability decreased in large seeds which at the same time had lower oil content. Although a general trend was visible, the relationship was between seed mass and oil content not significant (pMCMC= 0.16, posterior mean= -0.15, Figure 4A, left panel). Oil composition, i.e. UFA/SFA ratio, was also not significantly correlated with seed mass (pMCMC= 0.5, posterior mean= -0.03, Figure 4A right panel).

#### Seed longevity

Seed longevity was analysed using two different approximations, firstly using the raw germination data from the artificial ageing protocol (with MCMC-GLMM) but also using the p50 value (obtained from the probit analysis). P50 values ranged from 3 to 47 days, with a mean of 21 days (se=2). Results were consistent using both approximations, although raw germination scores had higher statistical power. Higher oil content significantly reduced seed longevity (germination curves pMCMC<0.001, posterior mean=-0.47; p50 p.value = 0.018 Figure 4B left panel). The signal for ratio was only marginally significant, seeds with higher UFA/SFA ratio (i.e. more unsaturated fatty acids) showed a decrease in longevity (germination curves pMCMC= 0.055, posterior mean=-0.28; p50 p.value = 0.35 fig 4B right panel).

#### Germination timing

T50 values ranged from 4 to 295 days with a mean of 150 days (se=16.6). We did not find any significant relationship between t50 and oil content (pMCMC= 0.13, posterior mean = 31.7, Figure 4C left panel) or UFA/SFA ratio, (pMCMC=0.1, posterior mean = 79, Figure 4C right panel). Despite the non-significance, we observed a trend of seeds with higher oil content and more UFA experiencing a delayed germination.

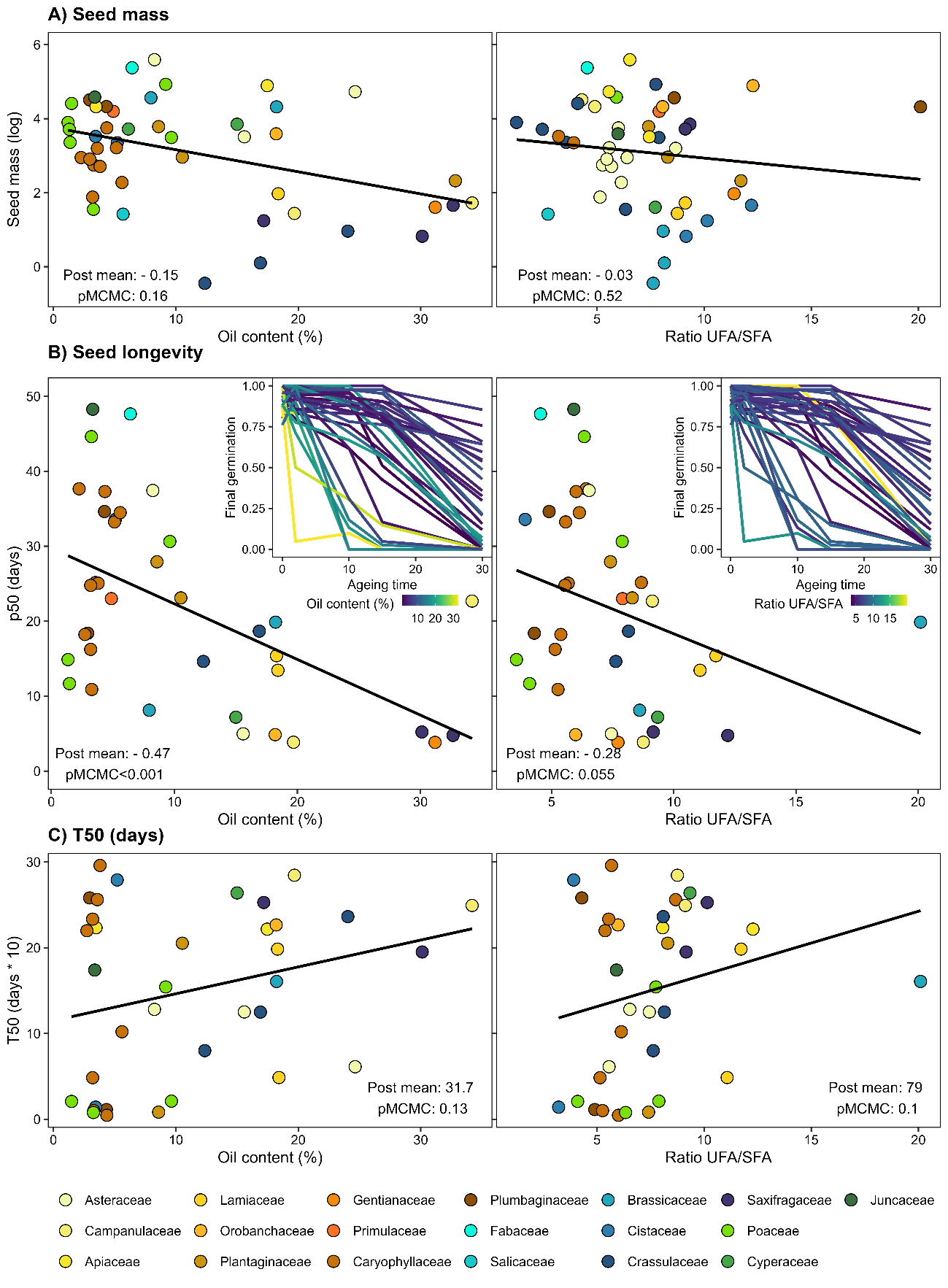


Figure 4. Seed oil content and Ratio UFA/SFA biological trade-offs. A) Correlation between seed mass and oil content) and ratio UFA/SFA, significances from MCMC-GLMM models (gaussian family, n= 47) with all variables log-transformed. B) P50: time for viability to drop to 50%, based on probit analysis (GENSTAT software) and the plot inset with raw germination curves across artificial accelerated ageing protocol in the lab, colour represents oil content percentage (left panel) and UFA/SFA ratio (right panel). Significances from MCMC-GLMM (multinomial family, N= 31) with oil content and ratio log transformed. C) Germination timing, T50: time to reach 50% germination, depending on oil content and ratio UFA/SFA. Significances from MCMC-GLMM (N= 36) both explanatory variables log-transformed. Colours represent the different families.

### Local ecological optimums

We found no significant correlations between species local optimal ecological conditions (GDD, FDD and Snow days, see Figure 5) and oil content or UFA/SFA ratios. GDD values ranged from 650 to 2295ºC with an average of 1421ºC (se=67.9), data showed a negative trend with species in warmer conditions showing less oil content and lower ratio. FDD values ranged from 0.05 to 170ºC with a mean of 31.8ºC (se=4.7), data showed a positive trend but likely driven by a few species with more extreme oil content and composition values. Snow values ranged from 3 to 157 days with an average of 62 days with snow, data showed a positive trend where species with a preference for more snowy sites show higher oil content and UFA/SFA ratio.

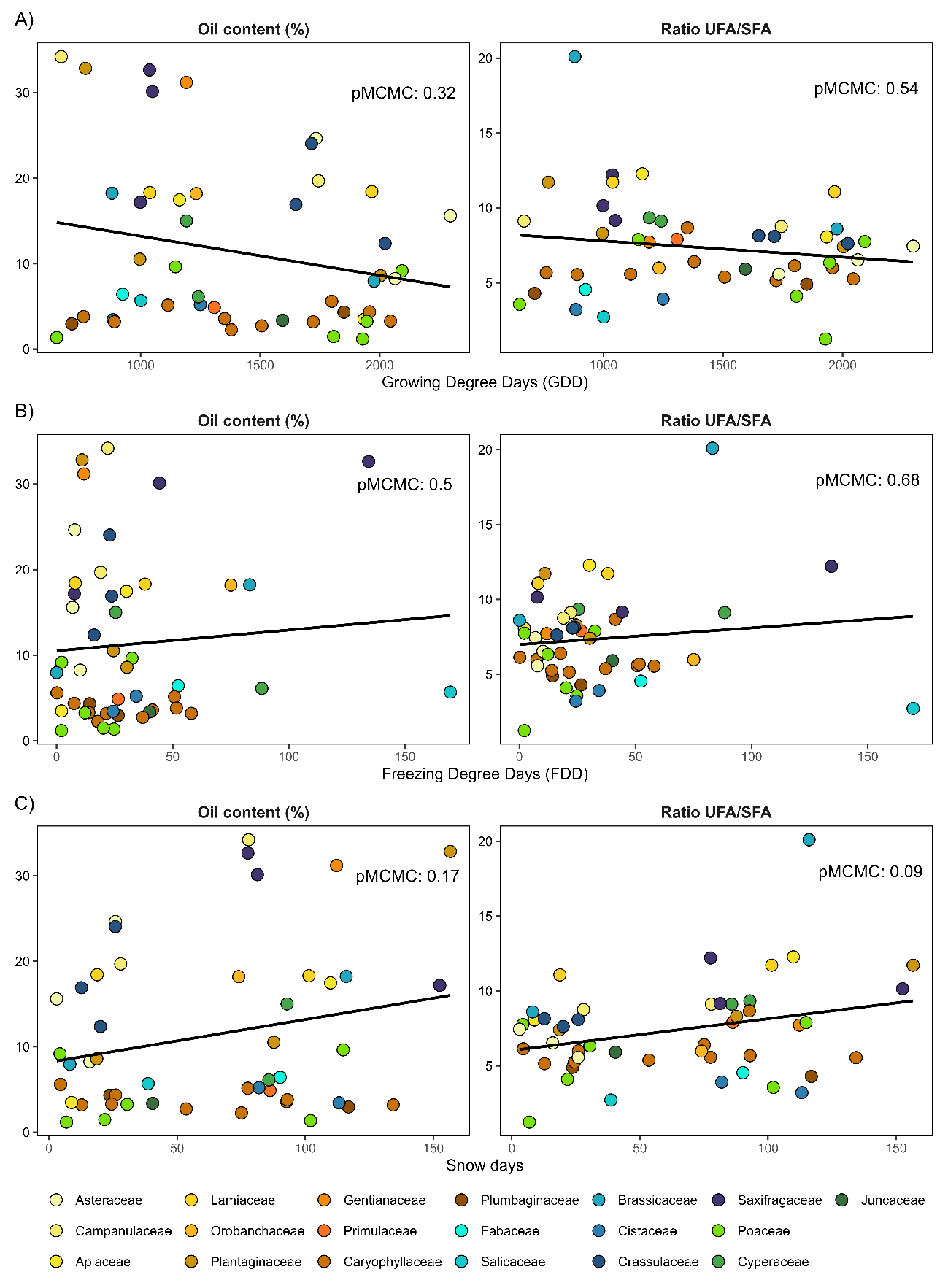


Fig 5. Seed oil content ecological trade-offs, oil content and ratio UFA/SFA log transformed for MCMC-GLMM models (family = Gaussian, N = 47). A) Relationship with GDD (Growing Degree Days ºC); B) Relationship with FDD (Freezing Degree Days ºC) and C) relationship with Snow days.

## Discussion

Our study is xxx

Oil content and composition in alpine species will show lower oil contents (is in the lower range of the reported in the literature) and higher UFA/SFA ratio than values reported in the literature (only 1 other study with this data and does not differ).

### Comparison with global patterns

Sanyal 2016 found significant negative correlation between seed oil content and palmitic (C16:0) and linoleic acids (C18:2n6) and positive correlation with oleic (C18:1n9), arachidic (C20:0) and eicosenoic (C20:1n9). In our preliminary analysis no significant high correlations between total oil content and other components in either direction.

The oil content can vary from 1 % in Musa paradisiaca to 76 % in Chrysobalanus icaco [9]. Bretagnolle found a huge variation in seed oil content ranging from seeds with virtually no oil (Trifolium pratense L., 0%) to very rich seeds (Papaver rhoeas L., 54%) (Bretagnolle 2016). In our preliminary results oil content varies from 1.3 % (F. glacialis) to 30.1% (S. conifera).

According to Bretagnolle 2016 most FA found in seeds are the saturated palmitic (16:0, PA) and stearic (18:0, SA) acids and the unsaturated oleic acid (18:1n-9, OLA), linoleic acid (18:2n-6, LA) and a-linolenic acid (18:3n3, ALA). Corroborated in our results + 2 extra FAs erucic (C22 1n9, mainly from Brassicaceae) and eicosenoic (c20. 1n9, mainly from Juncaceae and Brassicaceae).

Bretagnolle 2016. the three most common fatty acids were palmitic acid (C16:0, PA), oleic acid (18:1n- 9, OLA) and linoleic acid (18:2n-6, LA). These three FAs represented a mean of 76.5% (SD = 20.5%) of the total FAs in the seeds of the species analysed and can represent more than 90% of the FA synthesized by the seed. In our case also linoleic (18:2n-6, 43.1 ± 16.1%), oleic (C18:1n9, 22.9±13.3%), alpha-linolenic (C18:3n3, 16.2 ± 19.3%) and palmitic (C16:0, 10.6 ± 4%). These 4 represent a mean of 92.7% (SD = 52.75)

Two other FAs were found highly represented: the a-linolenic acid (18:3n3, ALA) and the c-linolenic acid (18:3n6, GLA). These five FAs (PA, OLA, LA, ALA and GLA) represented 85.7% of the FAs (SD = 13.1%) among all the species analysed, and for the majority of the species, these five FA’s represented more than 70% of the seed oil content. In our case, the next with higher % were stearic acid (C18:0, 2 ± 0.9%), c-linolenic acid (C18:3n6, 1.1±2.9%).

According to Bretagnolle 2016 the mean frequency of saturated fatty acids (SFA) was 15.6% (SD = 8.8%) and the ratio between unsaturated fatty acids (UFA) and SFA was 7.1 (SD = 4.3). In general, the Asteraceae and Poaceae had low values of the ratio, indicating the synthesis of a high proportion of SFAs, while other families such as the Apiaceae or the Brassicaceae were characterised by a high ratio indicating a tendency to synthesise mostly UFAs. In our case the mean frequency of SFA is 14.7% with SD 5.3, and the mean ratio between UFA and SFA is 6.8% with SD 2.1%. Ranging from 2.7 in salicacea up to 11.4 in lamiaceae.

‘unususal’ fatty acid species can be deposited in the seeds of particular angiosperm families that have evolved specific fatty acid modification and acylation pathways [8]. For instance, oil in Brassicaceae (Brassica napus, Arabidopsis thaliana) is rich in elongated acyl chains ranging from C20 to C24,(Baud 2010)

Erucic acid’s trend to lower levels at higher latitudes. In addition to having a relatively high melting point (33.5°C), erucic acid is also known to be unpalatable to many species of animals [29,42]. Since herbivory generally decreases at higher latitudes [43,44] selection might be stronger for higher levels of erucic acid at lower latitude.

### Biological trade-offs

Bretagnolle 2016 found a huge variation of oil content exists in small seeds, although such variation strongly decreases towards low oil values in large seeds (Corroborated in our preliminary results).

**Alpine species longevity or particularities in general?? Or keep for discussion??**

Differences between alpine and lowland seed lots are reflected at the genetic level by the rate or rearrangement of DNA and antioxidant responses during ageing (Donà et al 2013). Mondoni 2011 suggested that reduced longevity of seeds of alpine plants exposed to controlled ageing was caused by the low selection pressure for seed resistance to ageing, simple because seeds buried in the alpine soil are normally exposed to lower temperatures and therefore the rate of ageing are expected to be lower.

Waterworth 2010 showed that prolonged exposure of A. thaliana seeds to low temperatures resulted in reduced viability and vigour, due to increased oxidative stress under those circumstances. Warmer parental growth environment leads to a better seed provisioning in S. vulgaris through multiple effects, which promote and increased tolerance of the offspring seeds to heat stress.

### Ecological trade-offs

Oil content and UFA/SFA ration seems not to have an important/detectable ecological trade-offs at local scale.

We report the first evidence supporting adaptive evolution of seed TAGs in A. thaliana on a latitudinal cline and the first evidence that the plastic responses of seed TAGs to growing temperature appear to be adaptive. (Sanyal and Linder, 2013)

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