# Biological and ecological trade-offs of seed oil content in alpine species

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## Summary

## Introduction

Seed importance for plant regeneration, seed traits are understudied.

### Seeds reservoirs

Seeds act as reservoirs of energy basically accumulating 3 macromolecules: protein, lipids (triglycerides of fatty acids, FA) or carbohydrate (starch) (Levin, 1974). The quantity and quality of these reserves influence dormancy, viability and germination potential, thus directly related to plant fitness (Westoby et al., 1992. Fatty acids and carbohydrates are predominant for angiosperms (Bretagnolle *et al.*, 2016), but plants mostly rely on oil content, as has already been shown positively correlated with the earliness of germination (REF). Seed oil content and fatty acid composition thus determine plant fitness and validate the adaptive hypothesis, i.e. these traits are crucial for plant adaptation (Sanyal and Decocq, 2016), germination success (Linder, 2000), emergence and establishment of a plant [Bewley 1994]. Carbons in fatty acids are highly reduced and the oxidation of oils in germinating seeds releases more than twice as much energy as the oxidation of storage carbohydrates or proteins on a per g basis (Levin, 1974) (Baud & Lepiniec, 2010; Luttge, 2012; Theodoulou & Eastmond, 2012). Nevertheless, is not only the amount of oil but also the lipid composition which determines oil quality and membrane structure and has profound effects on seed viability in the dry state (Hoekstra, 2005).

### Seed oil composition (importance)

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). Both oil content and relative FA frequency vary as response to environmental, geographical location and maternal genotypes (Linder, 2000) Ghebretinsae et al., 2008). 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) (REF). FA can be divided in saturated (SFA) and unsaturated (UFA) which have differential properties that may influence those responses to biological and ecological trade-offs. On a per-carbon basis, UFA cost more to produce and yield less energy when oxidized than SFAs [41,(Linder, 2000). Moreover, the storage of unsaturated FAs and particularly PUFAs necessitates the storage of antioxidant molecules to prevent damage to FAs. Hence, the oily seeds should maximise SFA storage instead of UFA (Linder, 2000). Hence, a maximal storage strategy would be oriented towards the synthesis of oily seeds with only saturated FAs. However, the relative abundance of unsaturated to saturated FAs highly varies in angiosperms and many species synthesise a very low amount of saturated FAs (Linder, 2000)(Voelker & Kinney, 2001).

### Global seed oil content patterns

(Levin, 1974) found that seed oil content increased with woodiness and shade toleranceand was also lower in herbs than in shrubs than in trees but no geographical diffrerences between temperate, subtropical and tropical. However, a more recent study by (Sanyal and Decocq, 2016) found that seed oil content has been seen to be significantly higher in tropical plants, probably because need higher energy reserved in seeds to survive first life stages with high competition and/or low illumination (Levin, 1974) (Salisbury 1942), compared to temperate plants. Also, higher proportions of UFA (oleic and eicosenoic) with increased latitude (Sanyal and Decocq, 2016). Concordantly previous studies have shown that the proportions of saturated and unsaturated FAs and subsequently their melting points vary with latitude (Linder, 2000) 40]. At lower latitudes, seeds with higher proportions of saturated oils would be favoured because they would have more energy for growth without delaying or slowing germination. At higher latitude and cooler germination temperatures, seeds that have a higher proportion of unsaturated oils (with lower melting points) may germinate earlier and/or more rapidly than seeds that are higher in saturated FAs (with higher melting points). Rich UFA seeds could then germinate faster and earlier than rich SFA seeds in cold conditions, providing a competitive advantage where cold temperature regulates seed germination (Linder, 2000).

### Biological tradeoffs (seed longevity, seed mass, other germination traits?)

(Sanyal and Decocq, 2016) suggests that selection could be acting simultaneously on multiple seed traits: seed size, oil content and seed oil composition to facilitate faster growth and reproduction and higher latitudes or lower temperatures.

### Seed mass and oil content

(Bretagnolle *et al.*, 2016) found a negative correlation between seed mass and oil content (as well as (Levin, 1974) and a strong positive correlation between the energy stored in the seed and oil content. Large seeds generally store less oil than small seeds, whereas small seeds which have higher oil content. This relation makes sense because the carbons in fatty acids are highly reduced and the oxidation of oils in germinating seeds releases more than twice as much energy as the oxidation of storage carbohydrates or proteins on a per g basis (Baud & Lepiniec, 2010; Luttge, 2012; Theodoulou & Eastmond, 2012). Hence, a small oily seed can release as much as energy as a starchy seed that is twice as heavy. 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 preliminar results). 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 energy in a smaller volume (Bretagnolle *et al.*, 2016).

### Seed size and persistence

Small-seeded species often produce numerous seeds with higher persistence capacities, whereas large-seeded species often produce less but bigger seeds that generate seedlings with higher competitive ability and better buffering capacities of environmental stresses (Thompson et al., 1993; Coomes et al., 2002; Moles & Westoby, 2006; Muller-Landau, 2010).

### 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 and environmental factors. The genetic factors are associated with seed morphology and composition, whereas the environment affects by a combination of conditions prevailing during seed development, ripening, at harvest and during storage. Walters et al. (2005) suggested taxonomic and climatic effects on interspecific differences in longevity. According to these authors, seeds of 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). According to Mondoni et al. (2014) a relationship between seed longevity and the environment was considerably strong and greatly affected by maternal genetics. But according to Merritt et al. (2014b) environmental conditions of seed origin or production are weakly associated with seed longevity. On the other hand, according to (Probert, Daws and Hay, 2009) seeds from more stressful environments, as hot and dry, are more tolerant to desiccation and from moist and colder are more susceptible.

It is believed that lipid peroxidation is the main cause of seed deterioration in dry seeds during storage, which in turn influences longevity (Bewley et al., 2013). Stored lipids within the seeds deteriorate mainly due to (i) oxidation caused by high temperature and moisture content, (ii) hydrolysis, wherein fat is degraded into fatty acids and (iii) contamination (Abdellah and Ishag, 2012). To prevent the deleterious effect of lipid oxidation, many lipophylic antioxidants such as tocopherols and carotenoids are also stored in oily seeds, and a positive correlation has been shown between the tocopherol level and the degree of unsaturation in FAs (Kamal-Eldin & Andersson, 1997; Sattler et al., 2004; Falk & Munn\_e-Bosch, 2010). Seeds that contain a high concentration of lipids are susceptible to generate reactive oxygen species (ROS) during ageing that are responsible for cellular damage (Yao et al., 2012). Supporting evidence was originally based on the finding that auto-oxidation of polyunsaturated fatty acids produces free radicals, thereby compromising membrane integrity (Priestley and Leopold, 1979). Thus, oily seeds being more sensitive to ageing (Nagel and Börner, 2010). Accordingly, (Neto *et al.*, 2019) found a significant negative correlation between seed longevity and oil content. 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). In other cases, no significant effect of oil on seed longevity has been reported (Probert, Daws and Hay, 2009; Gardarin *et al.*, 2010) (Mederios et al 1998, Pritchard and Dickie lab not published)

In particular, the oxidative resistance of the oil decreases with increasing proportion of Polyunsaturated fatty acids (PUFA). Thus, the concentrations of UFAs and SFAs affect the storage behaviour of seeds (Walters et al. 2004; Volk et al. 2006; Walters et al. 2015). Oxidations of unsaturated fatty acids are considered to be primary reactions in ageing, contributing to free radicals production and subsequent attacks on other macromolecules (Benson 1990). Higher values of linolenic acid (UFA) associated with shorter longevity (Ponquett et al 1992). Further investigations on the effects of seed oils (content and composition) on longevity are desirable (Pritchard and Dickie, 2003).

Although its importance very few studies on native species address and analyze seed oil content and composition. A lot of specific studies in commercial species (cotton, brassica, soybean) gene expression, and irrigation effects (focused on plant breeding programs).

### Ecological significance (species optimal microclimatic conditions)

### Aims, questions and hypotheses.

The goal of the study is to explore the pattern of seed oil content and composition (proportion of unsaturated fatty acids, UFA and saturated fatty acids, SFA) in alpine plants and understand their biological and ecological trade-offs. This kind of data is barely available for most wild plants and especially for alpine species. Although oil content is known to influence a wide range of biological processes, few studies have investigated biological and ecological correlates. The trade-offs identified in the literature have not been consistent across showing divergent patterns. Our study is structured in 3 main sections/questions:

1. Exploration and description of seed oil content in alpine species. Does oil content and composition in alpine species follow global patterns?
2. Seed oil content biological trade-offs. How do oil content and oil composition (SFA vs UFA) correlate with other seed traits? Explore seed mass, seed longevity (p50) or other traits from previous experiments like t50?
   1. H2.1. Higher seed mass will correlate with less oil content (corroborated \*) and less UFA/SFA ratio (corroborated but marginal).
   2. H2.2. Less longevity will correlate with higher oil content (corroborated \*) and a higher UFA/SFA ratio (trend but NS). optimize space for more energy reserves
   3. H2.3. Faster germination (lower t50) will correlate with higher oil contend (opposite trend and NS) and higher UFA/SFA ratio (opposite trend and NS).
3. Ecological trade-offs. How does oil content and composition (UFA/SFA) correlate with the ecological optimal of the species/community?
   1. H3.1. Strict alpine species (specialists) will have less oil content (opposite and NS) and a higher UFA/SFA ratio (opposite and NS).
   2. H3.2. Species living preferentially in warmer places (+GDD) will have higher oil content (trend but NS) and higher UFA/SFA ratio (trend but NS).
   3. H3.3. Species living preferentially in colder places (+FDD) will have higher oil content (NS) and higher UFA/SFA ratio (opposite trend NS).

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

## Methods

### Study system

We focused on alpine species from grassland communities in the Cantabrian mountains (Northwestern Spain). These grassland communities are continuously distributed along the mountain range, occupying reduced areas above the treeline and around mountain tops, between 1750 and 2500 meters (figure 1). Grassland communities are mostly dominated by *Poaceae* and *Cyperaceae*, the main lifeforms are Hemicryptophytes and Chamaephytes and local richness ranges from 4 to 28 species. Grazing impact is restricted to wild populations of Cantabrian chamois (*Rupricapra pyrenaica parva*). The mountain hub is a transitional region between the Eurosiberian and the Mediterranean biogeographical regions and concordantly there is a clear climatic gradient from northern slopes (temperate) to southern slopes (Mediterranean).

Gráfico

Descripción generada automáticamente con confianza media

Figure 1. Distribution of plot data stored in a database on alpine grasslands in the Cantabrian Range, and mountain areas (M1, M2) selected for field studies. Grey areas show altitudes >1600 m. Mapa con capa de precipitación/temperauras? + cross design? Variation of temperatures measured by iButtons?.

### Species data

We established eight sampling sites, four in the northeastern sector and 4 in the southwestern sector. To cover spatial variation of community composition, in each sampling site we established 20 additional plots (1m2), five in each cardinal direction with a 10 m separation (cross design, fig x). In total, we established 160 vegetation plots (8 sites x 20 plots) where we collected floristic and community composition data, for a total of 128 alpine grassland species.

All species were classified between specialist (strict alpine) and generalist distribution. We identified plant specialists as those that are significantly associated with the target vegetation type, 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 values of elevation below 1800 m, most of them characteristic of subalpine or nitrophilous habitats. We finally identified 40 plant specialists which are widely recognized as characteristic species of alpine and subalpine grasslands.

### Oil content and composition.

We preliminary explored within-accession seed oil content variation in 5 species analysing 3 subsamples for each of those (also to ensure high precision of methodology), and we could not detect any statistical differences between subsamples. Consequently, a single sample for each species seed accession was analysed, so that within-species seed oil variation was not explored. Fatty acids (FA) analysis (oil content and composition) requires a sample of 200 mg of dry seeds we collected from the sampling sites. We collected enough seeds from 36 species, many of the data obtained about FAs content and composition of these species were not known before. 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) using a mix of sodium methoxide 0.5M in methanol and acetyl chloride in methanol (1:10 v/v) (table xx in appendix with complete list of Fame’s possible to identify). As a result, we obtained the oil content on a percentage of dry-weight bases and the percentage of each FAME’s type relative to the total of oil content.

### Seed trait data

Seed mass data was calculated for all species weighting 5 replicates of 50 dry seeds. Earliness of germination (T50 trait) is calculated as the time (days) to reach 50% germination and obtained from a phenology germination experiment (Espinosa del Alba et al. 2024), is available for 28/36 all species. under snow germ trait 36/36??.

Longevity data (raw germination scores and p50) is available for 20 of those 36 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) like alpine species (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 in 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 (*S. oppositifolia*), after removing from ageing conditions were put into a refrigerator at 5ºC and darkness for a month before starting germination experiments (same procedure followed by (Satyanti *et al.*, 2018). In some species, the number of seeds for subsamples was reduced due to the lack of seeds. Germination conditions for the species were set at 22-12ºC alternating temperatures with a 12/12 h photoperiod or for those whose germination required colder temperatures a second germination chamber was set at 15/5ºC alternating temperatures with a 12/12h photoperiod (see table xx for details). After the sowing, the seeds were checked once a week for one month. 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 days germination test, the ungerminated seeds were cut-tested under the binocular loupe 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.

Longevity analysis have been widely used by seed bank managers based on the viability equation: 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.

### Bioclimatic indices

To calculate species ecological optimums (following the methodology (Jiménez-Alfaro *et al.*, 2024), we measure microenvironmental gradients using 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. In basic plots, the iButtons recording period went from 1st October 2018 to 31st August 2019 (330 days) while in acidic vegetation plots, the recording period for the iButtons went from 12th July 2021 to 29th May 2022 (321 days, all raw data available in GitHub repository).

From the environmental data, we calculated a series of bioclimatic indices per plot following (Jiménez-Alfaro *et al.*, 2024). We calculated bioclimatic indices based on standard variables used by WorldClim (Fick & Hijmans 2017), together with other variables with a relevant function on alpine topographic gradients. The selected variables were: (1) bio1 = annual mean temperature; (2) bio2 = mean diurnal range, i.e. the mean of the monthly differences between maximum and minimum temperatures; (3) bio7 = temperature annual range; i.e. the difference between the maximum temperature of the warmest month and the minimum temperature of the coldest month; (4) snow = the number of days of snow cover, when temperature is around 0 ºC, calculated for the period in which the maximum temperature was < 0.5 ºC and the minimum temperature was > -0.5 ºC; (5) FDD = freezing degree days, i.e. the sum of daily mean temperatures for days in which the mean temperature was below 0 ºC (Choler 2018); and (6) GDD = growing degree days, i.e. the sum of daily mean temperatures for days in which the soil mean temperature at five cm deep was above 5 ºC (Körner 2021). For FDD, we transformed the values from negative to positive, so higher values represent more freezing. Then we use the presence and abundance of each species in each plot to estimate an adult optimal ecological niche. For each species we considered those plots with more than 1% of abundance and….CHECK!!!

### Statistical analysis

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

Phylogeny was included using a reconstructed tree for the 36 species (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).

Exploratory PCA for FAME’s composition and total oil content (in percentage)

Our two response variables are total oil content (in percentage) and UFA/SFA ratio (both log transformed).

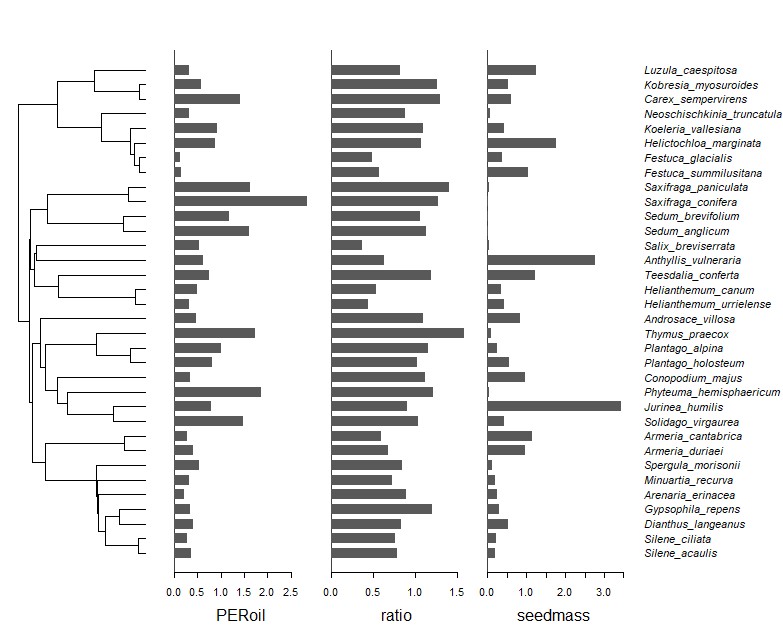
Biological trade-offs: seed mass, longevity (p50), earliness of germination (t50).

MCMC-GLMM: 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). When needed traits were log transfomed to ensure 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).

Ecological trade-offs: species ecology, GDD, FDD

## Results

Congeneric species has very similar levels and proportions of FA and oil content (as stated in literature, and see figure 2)



### Seed oil content patterns in alpine species

The exploratory PCA showed relatively low explained variation within the first two axes (21.7 and 16.2 5 respectively). FAMEs of C22:1n9 and C22:2n6 are the ones contributing the most in PC1 and PC2 (around 11%) along with the percentage of oil content (11%) also contributing in PC2 (Fig 3A and 3B). Additionally, PCA revealed that oil content percentage is not highly correlated with any specific FAMEs type (table in appendix).

Within our species, oil content varies from 1.3% in *Festuca glacialis* (Poaceae) to 30.1% in *Saxifraga conifera* (Saxifragaceae) (Fig 3C). Most abundant FAME in alpine seeds were the unsaturated linoleic acid (18:2n-6, LA, 43.1 ± 16.1%), oleic acid (18:1n-9, OLA, 22.9±13.3%) and alpha-linolenic acid (18:3n3, ALA, 16.2 ± 19.3%); and saturated palmitic (16:0, PA, 10.6 ± 4%). These 4 represent a mean of 92.7% (SD = 52.75). The next with higher % were stearic acid (C18:0, 2 ± 0.9%, saturated) and c-linolenic acid (C18:3n6, 1.1±2.9%, unsaturated). Additionally, in our data set, Erucic acid (C22:1n9) had also high values but only in Brassicaceae species (Fig 3D). The mean frequency of saturated fatty acids (SFA is 14.7% with SD of 5.3) and the mean ratio between unsaturated fatty acids (UFA) and SFA is 6.8 (SD= 2.1), ranging from 2.7 in *S. breviserrata* up to 11.4 in *T. praecox*. In general, the Salicaceae (*S. breviserrata*) and Cistaceae (*H.canum* and *H.urrielense*) had low values of the ratio, indicating higher synthesis SFAs, while other families such as the Lamiaceae (T. praecox) or the Saxifragaceae (*S. conifera* and *S. paniculata*) were characterised by higher ratios showing a tendency to synthesise more UFAs (fig 3E).

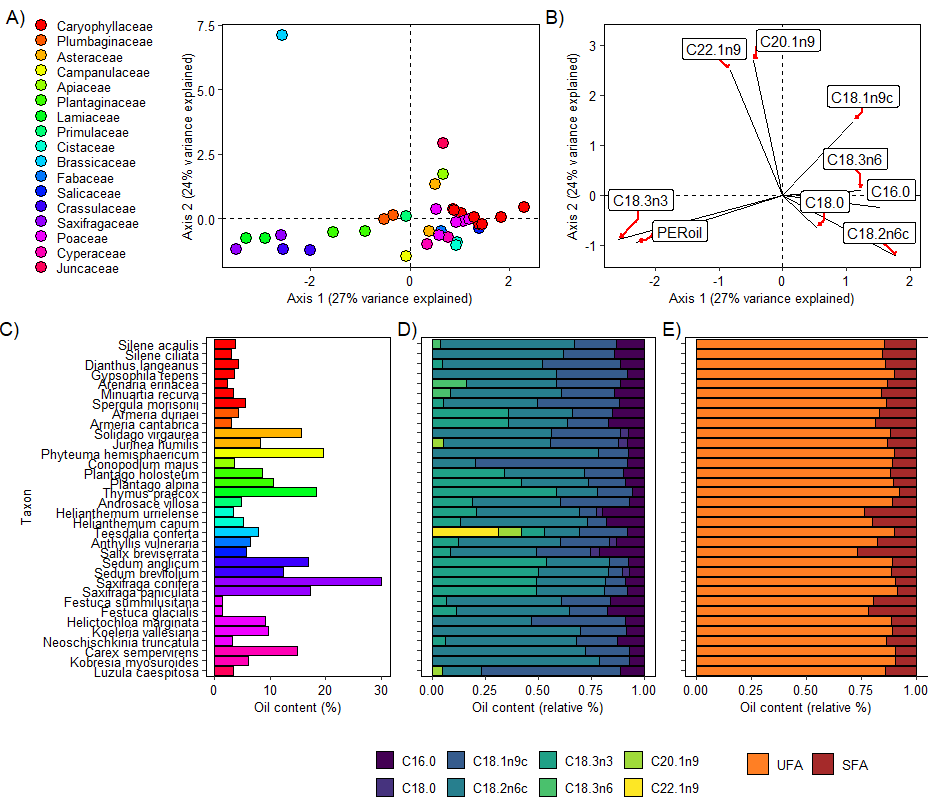


Fig 3: Alpine species seed oil content and composition exploration (n=34). A) Exploratory PCA species points and B) variables directions and contributions. C) Oil content per species in percentage, colors representing families as panel A. D) Seed oil composition of those FAME’s with more than 3% of relative abundance. E) Seed oil composition per species with FAME’s divided between Unsaturated Fatty Acids (UFA) and Saturated Fatty Acids (SFA).

### Biological trade-offs

#### Seed mass

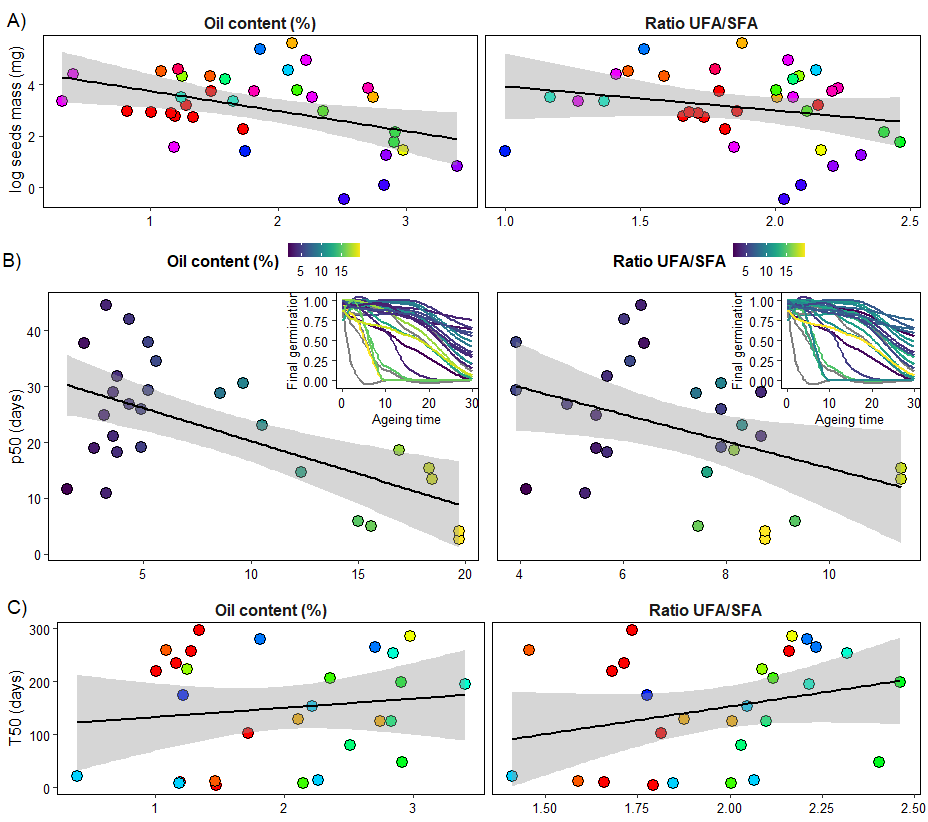
Seed mass values (dry weight 50 seeds) ranged from 0.6 to 268 mg, with a mean of 47.5 mg (sd=58.8). We observed a notable variation of oil content in small seeds, but its variability decreased in large seeds which at the same time have lower oil content. We found a significant negative correlation between seed mass and oil content (p<0.05, fig 4A, left panel) and a marginal negative relationship between seed mass and UFA/SFA ratio (p = 0.06, fig 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 45 days, with a mean of 22 days (sd=11). Results were consistent using both data types, although raw germination scores had higher statistical power. Higher oil content significantly reduced seed longevity (p<0.05, fig 4B left panel) and higher UFA/SFA ratio (i.e. more unsaturated fatty acids) also decreased longevity, although the signal found in the ratio was only marginally significant (p= 0.08, fig 4B right panel).

#### Earliness of germination

We measured the earliness of germination (germination speed) using t50 value from Espinosa del Alba et al (2024), who worked with a similar dataset of species. T50 values ranged from 4 to 295 days with a mean of 149 days (sd=102). We did not find any significant relationship between t50 and oil content (fig4C left panel) o UFA/SFA ratio, although it appears to be a trend (+ ratio – t50 ) with the latter (fig 4C right panel).

Fig 4. Seed oil content and Ratio UFA/SFA biological trade-offs. A) correlation between seed mass (log-transformed) and oil content (% log-transformed) and ratio Unsaturated Fatty Acids (UFA)/ Saturated Fatty Acids (SFA), also log-transformed. Colours represent families as in Figure 3) P50: time for viability to drop to 50%, based on probit analysis (GENSTAT software) and raw germination curves across artificial accelerated ageing protocol in the lab, colour represent, oil content percentage (left panel) and UFA/SFA ratio (right panel). C)T50: time to reach 50% germination, data from germination phenology which uses the same species from the same area (Espinosa del Alba et al., 2024) depending on oil content (% log-transformed) and ratio UFA/SFA (log-transformed). Colours represent families as in Figure 3.

EXTRA???: will ecological preferences modify longevity? AS preliminary results from Giovanni tesis and IAVS congress. Significant only when using raw germination data.

Gráfico, Diagrama

Descripción generada automáticamente

### Ecological trade-offs

#### Species distribution

Within our 36 species, we had 24 classified as specialist (strict alpine) and 12 as generalist. We found no significant differences between these two groups in oil content or ratio UFA/SFA, nevertheless looking at the data specialist species appeared to have a wider range of values compared to generalists which have higher mean values of both oil content and ratio UFA/SFA (fig 5A).

#### Optimal ecological conditions

Species optimal ecological conditions in GDD ranged from 650 to 2295ºC with a mean of 1473ºC (sd=481). We found no significant relationship between GDD and oil content or UFA/SFA ratio, although it seems to follow a positive trend.

Species optimal ecological conditions in FDD ranged from 0 to 170ºC with a mean of 29ºC (sd=30). We found no significant relationship between GDD and oil content or UFA/SFA ratio, although it seems to follow a negative trend when looking at the ratio.

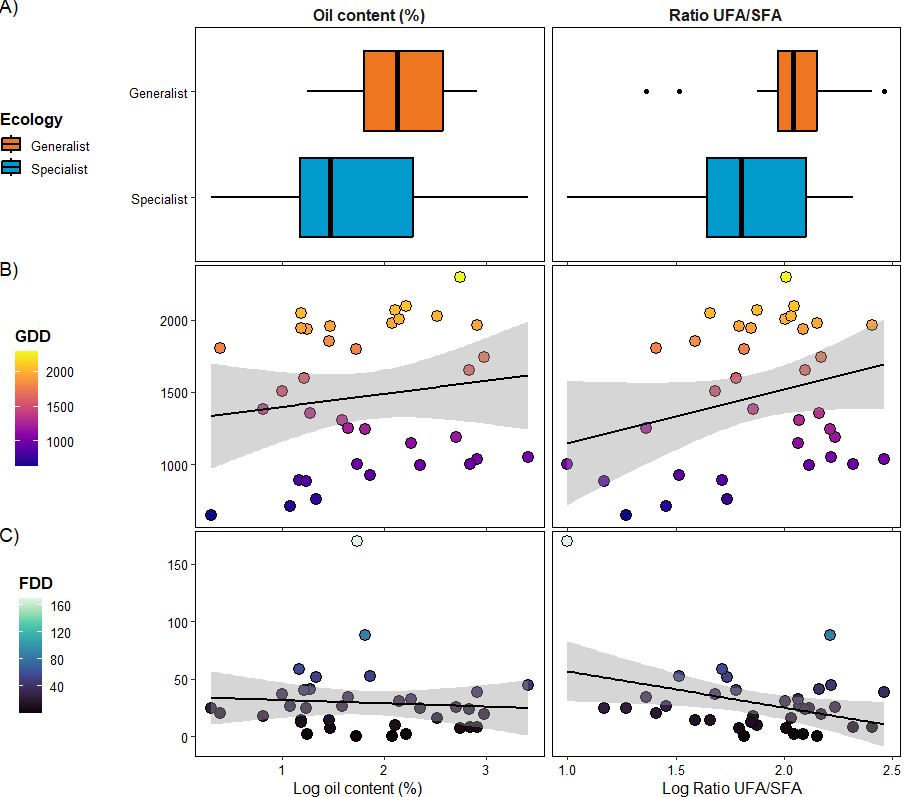


Fig 5. Seed oil content ecological trade-offs. A) Differences of oil content (%, log-transformed) and UFA/SFA ratio (log-transformed) between specialist and generalist species. B) Relationship between GDD (Growing Degree Days) and oil content (%, log-transformed) and UFA/SFA ratio (log-transformed). C) Relationship between FDD (Freezing Degree Days) and oil content (%, log-transformed) and UFA/SFA ratio (log-transformed).

## Discussion

Our study is xxx

### 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.

### 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).

### Ecological trade-offs

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

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