

**Can germplasm storage
compensate for habitat
loss? Handling the
conservation of the
priority species *Klasea
lycopifolia* (Vill.) Á.Löve &
D.Löve in central Italy**

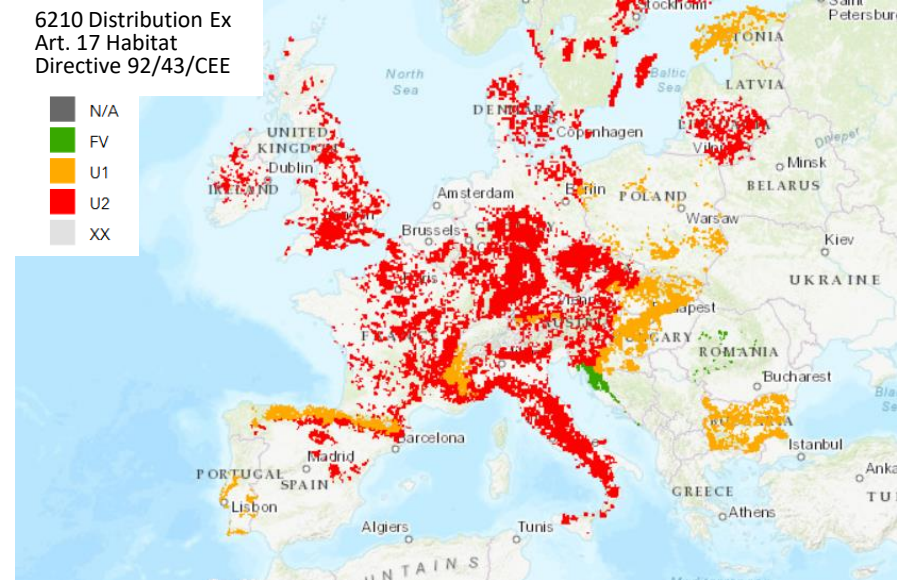
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DIPARTIMENTO
DI SCIENZE AGRARIE,
ALIMENTARI E AMBIENTALI

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Secondary grasslands in danger



SECONDARY GRASSLANDS INCLUDE MANY HABITATS OF EUROPEAN IMPORTANCE, LISTED IN ANNEX I OF THE HABITATS DIRECTIVE (92/43/EEC), SUCH AS 6210, 6220, 6230 AND 6510



THEY DEPEND ON **TRADITIONAL AGRO-PASTORAL ACTIVITIES**, SUCH AS MOWING OR LIVESTOCK GRAZING, FOR THEIR CONSERVATION



THE HARDLY REVERSIBLE ABANDONMENT OF TRADITIONAL AGRICULTURAL ACTIVITIES ENDANGERS SECONDARY GRASSLANDS AND ALL THE SPECIES THAT INHABIT THEM



IN-SITU AND EX-SITU CONSERVATION MEASURES FOR GRASSLAND SPECIES ARE NEEDED!

MOST OF HABITAT 6210 SURFACE IS CLASSIFIED AS “U2” – UNFAVOURABLE – BAD!

MS/EU28	Region	Curr. CS trend	Curr. CS
EU28	ALP	↘	U2
EU28	ATL	↘	U2
EU28	BLS	DD	U1
EU28	BOR	↘	U2
EU28	CON	↘	U2
EU28	MED	↘	U2
EU28	PAN	→	U1

Conservation status of hab. 6210 in the different biogeographic regions of Europe (ex. art. 17 Habitat Directive (92/43/EEC))

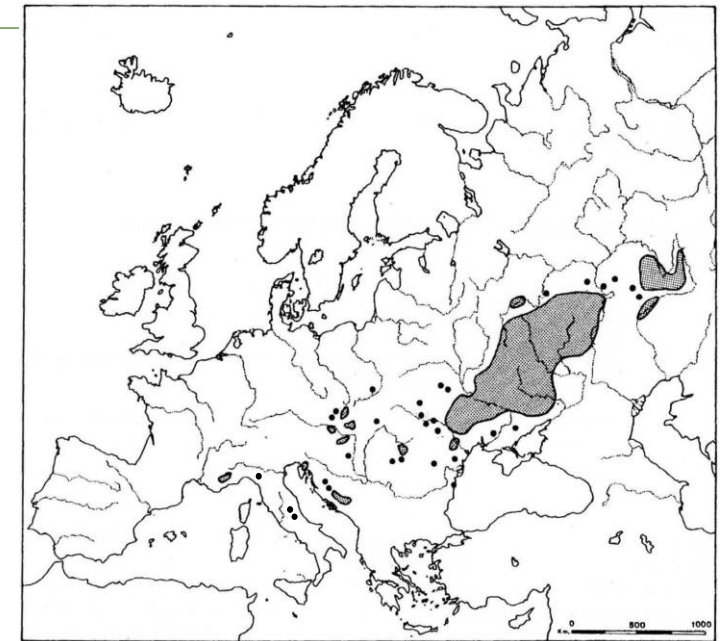
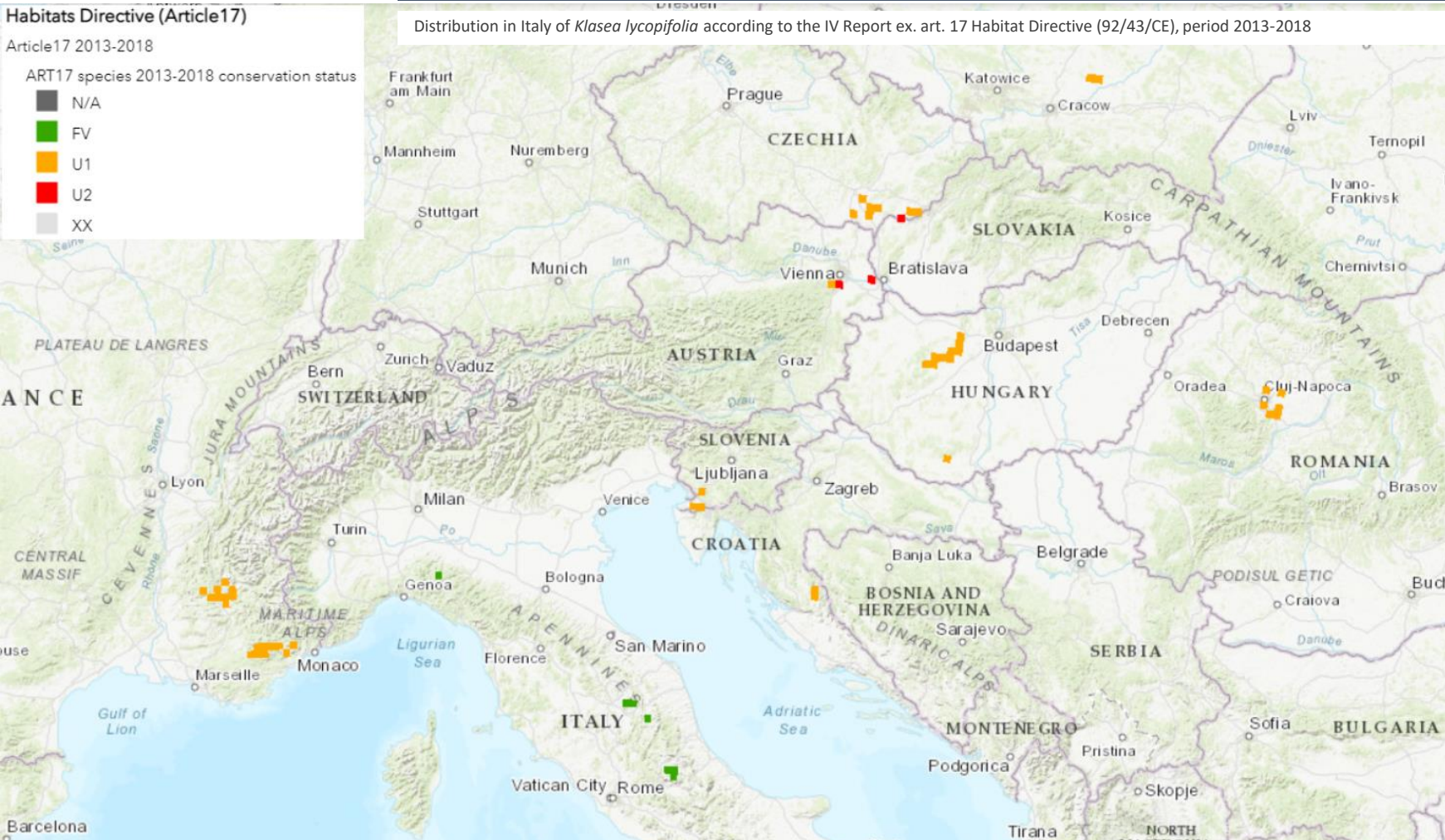
A case study: *Klasea lycopifolia* (Vill.) Á.Löve & D.Löve

- *Klasea lycopifolia* is hemicryptophytic perennial plant of the Asteraceae family, with an erect habit, endemic to SE-European mountain ranges and **strictly connected to secondary grasslands**
- listed as a priority taxon in the Habitat Directive Annexes II-IV (Dir. 92/43/EEC).
- Classified in the category "Nearly Threatened" (NT) of the Italian Red List of flora
- *In-situ* and *Ex-situ* conservation measures are needed!

Code	Kingdom	Class	Priority	Annex HD	EU Red List IUCN (*)	IT Red List IUCN (*)
6282	Plantae	Magnoliopsida	Yes	II-IV	DD	NT



Distribution of the species



Distribution of *Klasea lycopifolia* in Europe (modified from Meusel & Jager 1992 and Conti & Manzi 1997)

The species has a wide eastern-European range

The Italian sites represent the southwestern limit of the range and feature isolated and fragmented stations.

Aim of the study

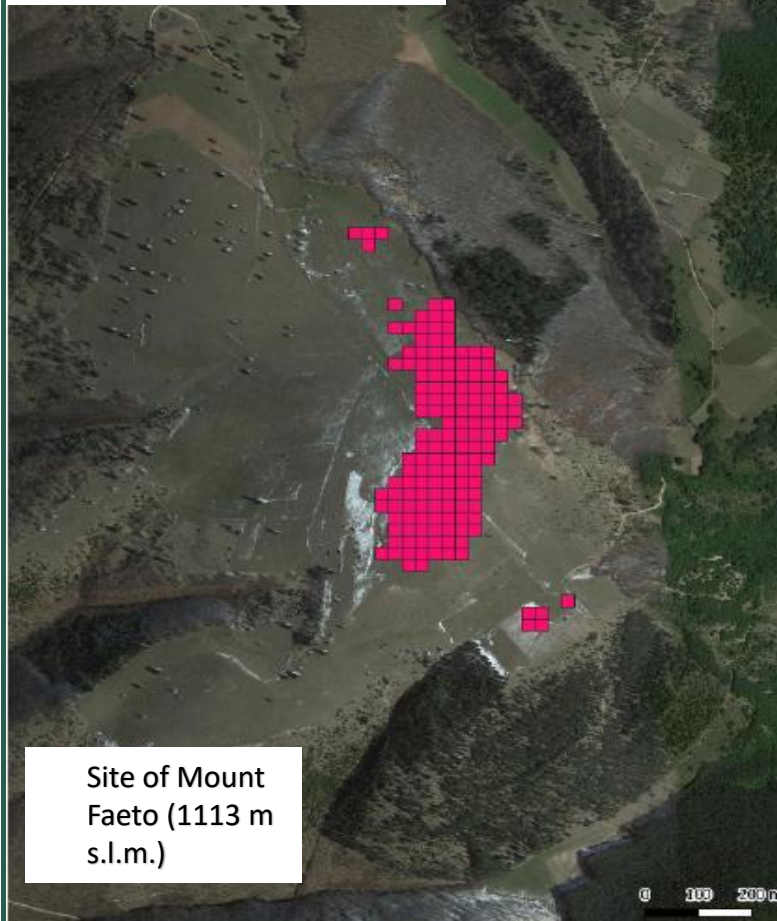
1. Assess whether anthropogenic variables such as Land management (mowing vs. grazing), and topographic variables such as slope and aspect affect the species and which of these affect it significantly, in order to understand how to best manage its *in-situ conservation*
2. Evaluate the role of *ex-situ* conservation approaches for *K. lycopifolia*
3. Confront the possible role of *in-situ* vs. *ex-situ conservation* in order to develop a comprehensive complementary strategy for *K. lycopifolia*

Study areas

We studied two populations in central Italy (Umbria): Mount Pennino and Mount Faeto. The two sites are represented by summit grasslands with the same Altitude range (1100-1150 m a.s.l.), Climatic characterization (Temperate, Submediterranean variant), Geological substrate (Limestone).

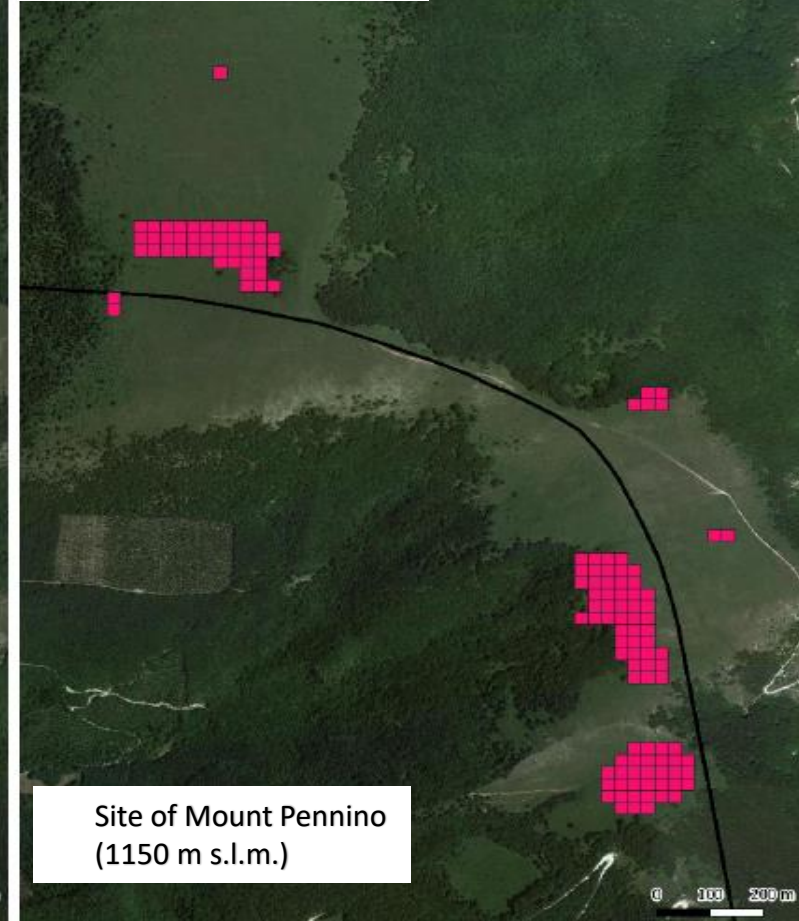


Surface covered by *Klasea lycopifolia* (cells of 25x25 m)



Site of Mount Faeto (1113 m s.l.m.)

Surface covered by *Klasea lycopifolia* (cells of 25x25 m)



Site of Mount Pennino (1150 m s.l.m.)

Materials and methods

- ❖ 2 survey campaigns: 2011 and 2017, in both presence sites: M. Pennino and M. Faeto
- ❖ Measurements conducted using transects formed by squares (40*40 cm) along the major axis of the colony



Measurement Transect

❖ Demographic parameters:

- ✓ Number of Ramets /m²
- ✓ Number of flowered stems/m²
- ✓ % flowered stems
- ✓ Length of colonies

❖ Morphological parameters:

- ✓ Stem height (cm)
- ✓ Height of floral envelope (mm)
- ✓ Diameter of floral envelope (mm)
- ✓ Length of floral peduncle (cm)

confronted
with...

Environmental drivers

❖ Geomorphological parameters:

- ✓ Slope
- ✓ Aspect (N/S)

❖ Land management

- ✓ Mowing
- ✓ Grazing

❖ Year (2011/2017)

Which of these
exerts significant
influence on the
population features?

Statistical analyses

The analyses have been carried out using the tool for statistical analyses Rstudio.

The following tests have been applied:

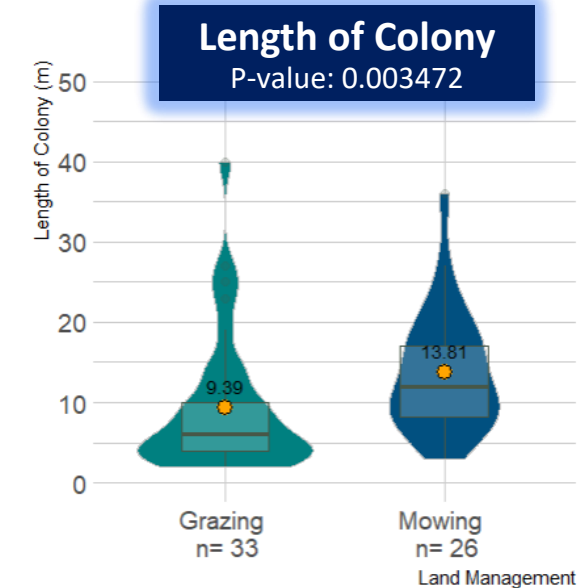
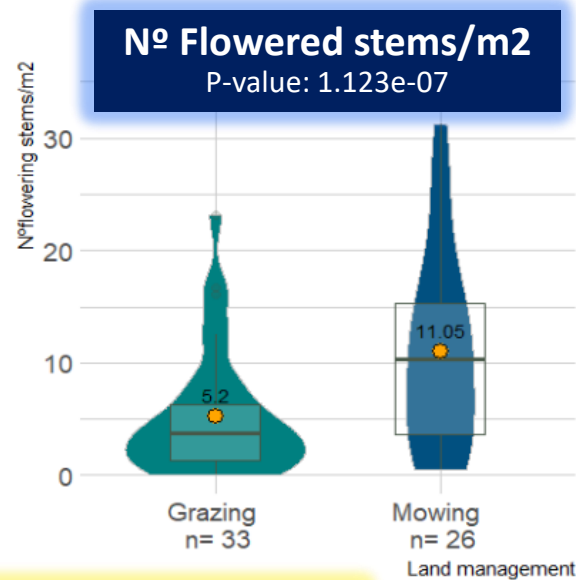
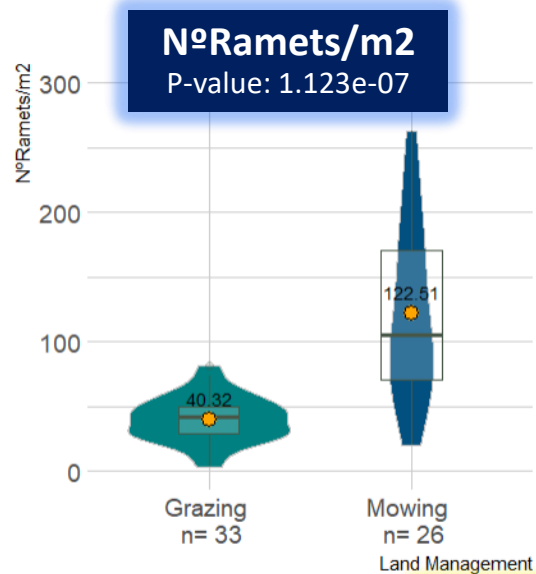
- ✓ **Shapiro-Wilk normality test:** to assess whether the data followed a normal distribution;
- ✓ **T-Student test:** to determine if there is a significant difference between couples of normal parameters
- ✓ **ANOVA:** to determine if there were significant differences between multiple groups of normal parameters,
- ✓ **Mann-Whitney U test:** to determine if there were significant differences between 2 groups of non-normal parameters;
- ✓ **Kruskal-Wallis test:** A non-parametric statistical test (for non-normal parameters) used to determine if there are significant differences between the parameters

Results have been thus represented in **violin-boxplot graphs**, to gain an immediate representation of the distribution of the observations.

Results: Land management



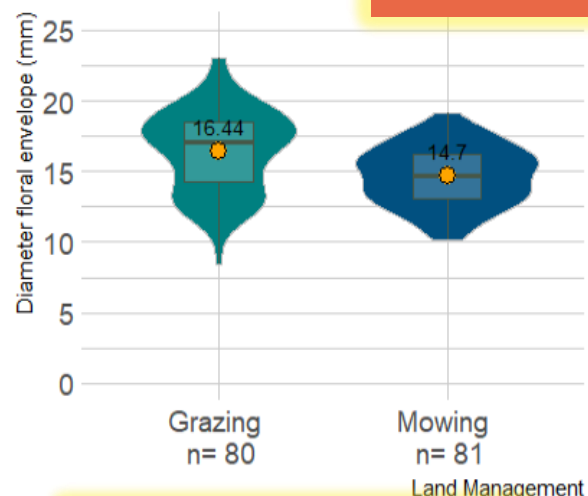
Demographic parameters



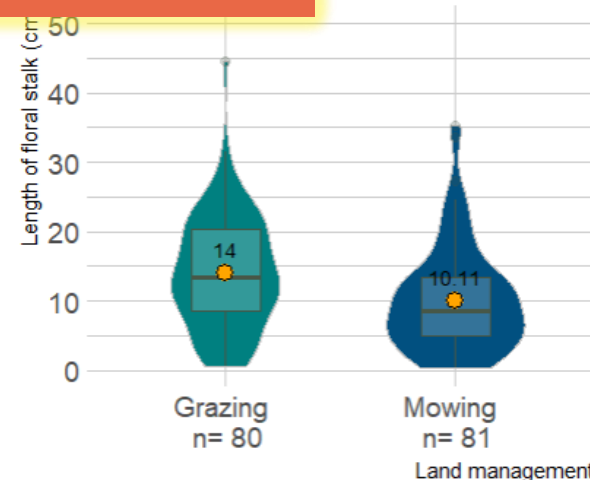
Morphologic parameters



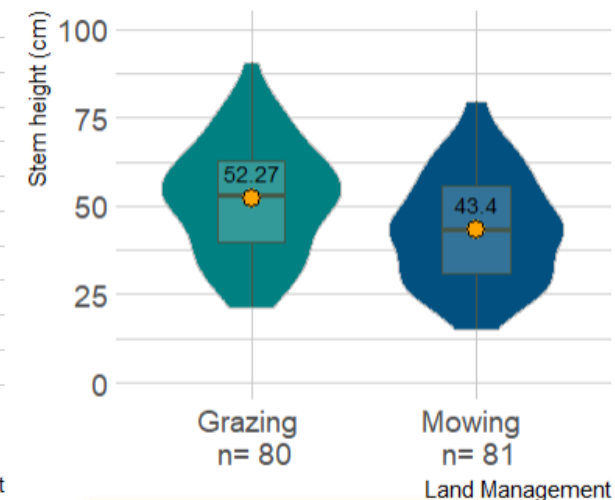
Height floral envelope
P-value: 0,003898



Diam. Floral envelope
P-value: 3,222e-05



Length floral peduncle
P-value: 0.0009119



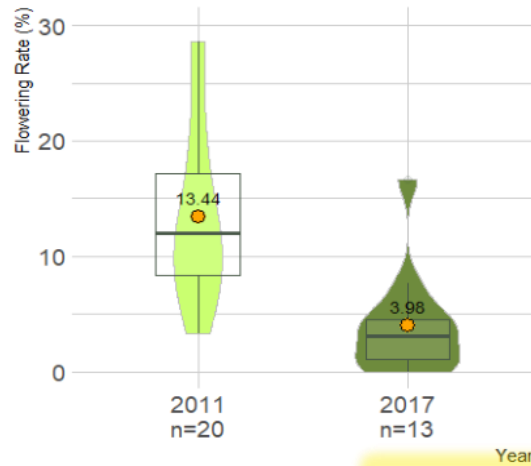
Stem height
P-value: 0,0004533

Results: Year



M. Pennino – Flowering rate

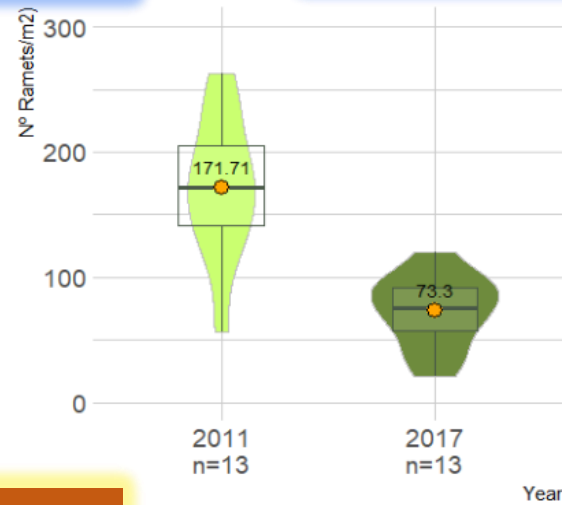
P-value: 4,67e-05



Demographic parameters

M. Faeto – N° Ramet/m²

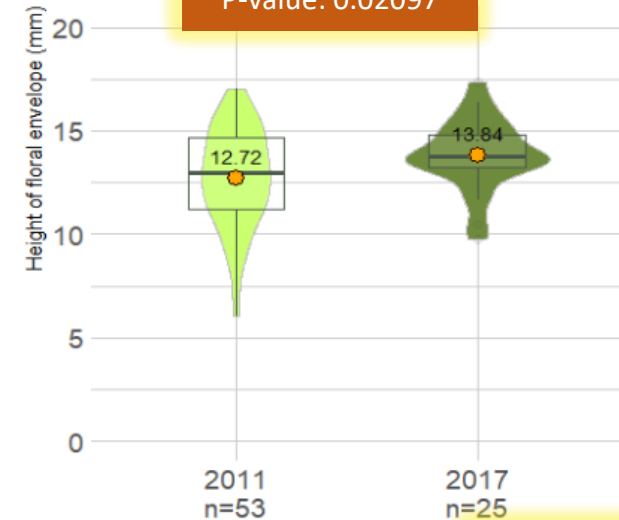
P-value: 3,75e-05



Morphologic parameters

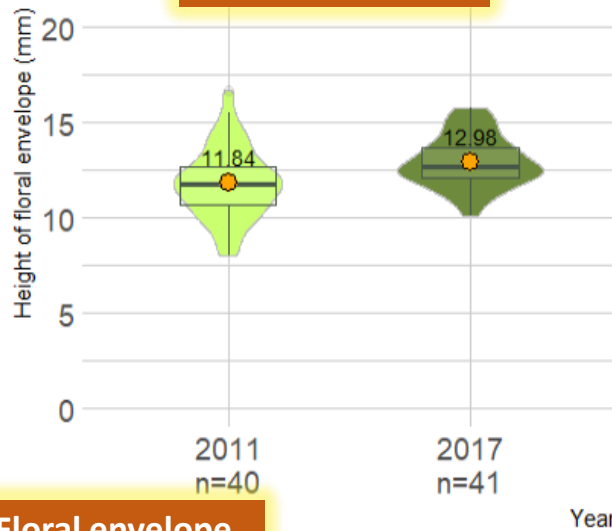
M. Pennino

P-value: 0.02097



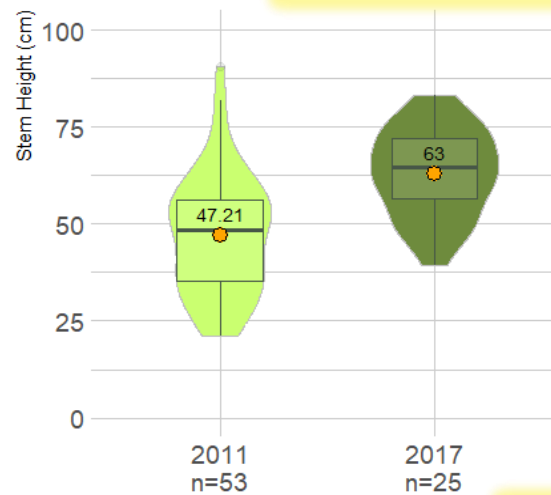
M. Faeto

P-value: 1,779e-08



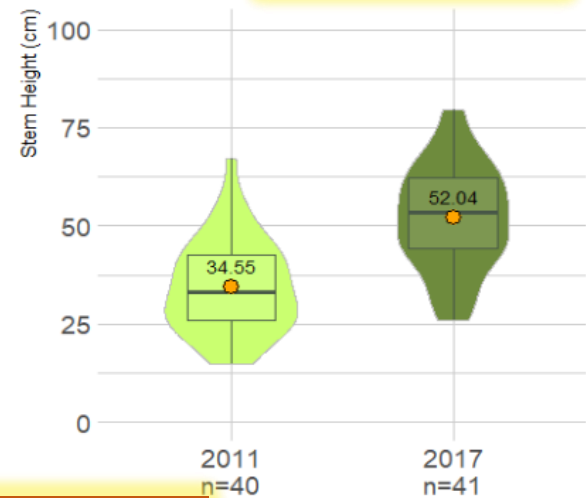
M. Pennino

P-value: 3,189e-06



M. Faeto

P-value: 1,779e-08



Height of Floral envelope

Stem height (cm)

Ex-situ conservation strategies

- ❖ **Significant Reproductive Changes:** Between 2011 and 2017, *Klasea lycopifolia* experienced a decrease in reproductive rate (M. Pennino) and population size (M. Faeto).
- ❖ **Importance of Ex-situ Conservation:** We don't know yet if this negative trend will continue. Anyway, considering also that both the sites of occurrence fall into private properties, thus not subject to any kind of protection, these populations of *Klasea lycopifolia* might be in danger.
- ❖ **Germplasm collection:** To this end, germplasm collections were conducted in 2011, 2017, and 2021. The collected material is now stored in the germplasm bank of the Department of Agricultural, Food and Environmental Sciences at the University of Perugia
- ❖ **Research Focus on Germination Biology:** The gathered material underwent germination tests, to gain a deeper understanding of the germination biology of *Klasea lycopifolia*.



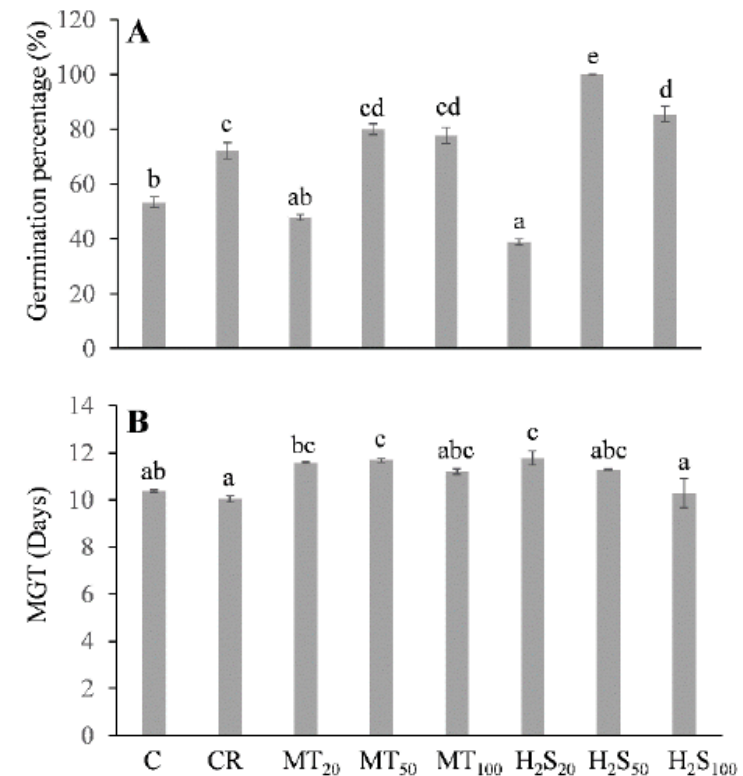
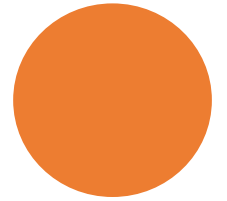
Klasea population in M. Pennino



Effect of *K. lycopifolia* achenes pre-chilling treatment and different concentration of Melatonin (MT) and Hydrogen sulphide (H₂S) on seeds germination rate and mean germination time (MGT).

- 3 replicates of 30 seed each for every treatment
- Pre-chilling treatment (CR): Achenes at 4°C for 100 days
- MT and H₂S Concentrations: 20,50,100 M
- Germination % and Medium Germination Time (MGT): Assessed on the 18th day, when 100% germination reached in at least 1 treatment
- Data represent the mean ± SE. Different letters indicate statistically different mean values (p ≤ 0.01; ANOVA one-way, Tukey's HSD tests).

- Germination percentages are >50% in almost all experimental treatments
 - MGT is for all the treatments around 10-12 days!
- Both Hydrogen sulfide and Melatonin positively affect seed performance during germination and seedling growth of *Klasea lycopifolia*!!
 - The best performance (100%) is reached with H₂S 50M



Conclusion

From the *in-situ* conservation point of view....

- Mowing appears to be the best technique to obtain bigger colonies, more numerous in terms of ramets and flowering stems (though more genetically homogeneous).
- Grazing seems to significantly increase the size of the individuals and their flowering heads.
- Between 2011 and 2017, a negative trend in population size was detected, a potentially worrying signal if we don't take action for the conservation of this species!
- The keyrole of private managers/owners of the areas should be enhanced and rewarded, for instance, with the instrument of payments for ecosystem services (PES)

From the *ex-situ* conservation point of view...

- The results of this study identified efficient pre-germination treatments to achieve maximum percentage germination, allowing the development of a standardized germination protocol

Given the current absence of specific conservation protocols about this species, these findings hold particular importance in shaping forthcoming conservation approaches for the priority Annex II-IV species *Klasea lycopifolia*.



Thanks for the attention!