Annex to Deliverable A4.2

"Scientific Report on recovery activities in Alta Murgia"

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Executive summary

This report is an Annex to Deliverable 4.2 and contains details about the starting of recovery nature-based activities in Alta Murgia study site in the framework of NewLife4Drylands.

As foreseen in the Agreement between CNR-IIA and Department of Biology, currently Department of Biosciences, Biotechnology and Environment (DBBA) of the University of Bari “Aldo Moro”, a scientific activity was carried out with the aim of providing design and technical-scientific support for the implementation of limited pilot experimental interventions for the restoration of grassland in Alta Murgia.

The scientific activity carried out is presented in this document, as required by art. 3 of the aforementioned Agreement.

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1. Scientific report on the activities

The process known as "stone removal", which consisted in clearing and crushing limestone rock, was practiced in Alta Murgia site between the end of the 20th and the beginning of the present century. This led to the elimination of many hectares of sub-Mediterranean xeric grasslands with strong trans-Adriatic affinities featuring a peculiar endemic character, known by the inappropriate term of "pseudo-steppe". These semi-natural herbaceous communities are particularly important from a naturalistic and a landscape point of view: in fact, among those of the Alta Murgia, they are the richest both in terms of animal and plant species and have the highest rate in biodiversity, which originated thanks to the grazing activities which were carried out in this territory for centuries (see, for example, the establishment of the Royal Customs for the management of sheep dates to the year 1447).

The landscape and naturalistic relevance of these meadow communities and the profound ecological system transformations triggered by stone removal and its consequences (i.e., the removal of rockiness, the loss of organic substance, and the increase in fine soil material), require a careful analysis of the natural evolutionary dynamic processes involved. In addition to this, *restoration* techniques need to be tested in order to achieve, none the least, an acceleration of the renaturalization process. A testing process proves to be even more necessary in Alta Murgia grasslands, because the current techniques from specialized literature were developed and are being used in areas which are ecologically different than the sub-Mediterranean Murgia.

As stated, the scientific activity conducted by the Department of Biosciences, Biotechnology and Environment of the University of Bari, within the framework of the Agreement with CNR-IIA, was aimed at providing design and technical-scientific support for the implementation of limited pilot interventions for the *restoration* of Alta Murgia grasslands.

As a first step, we located a suitable site for testing methodologies for an *in-situ* restoration of dry grasslands, attributed to ‘habitats of community concern” pursuant to Directive 92/43/EEC (Natura 2000 code: 62A0). The identified areas were localized among those pertaining the SAC IT9120007 ‘Murgia Alta’, which is located on the property of the ‘Masseria Modesti’, in the municipality of Ruvo di Puglia (Fig. 1), where stone removing was carried out in the past: they consist of two long strips of land, measuring 10 meters in width and 450 meters in length each, separated by a ca. 5-meter-wide band in their length. The two strips were selected as representative of two different forms of land use: the first strip, in fact, endured continuous ploughing after the stone removal, while the second strip had hardly seen any ploughing activities for the previous 3-4 years and a rather periodic mowing activity. Nonetheless, they are representative of two different moments in the recolonization’s natural dynamics of the stone-removed areas, with the first strip constituting time "0".

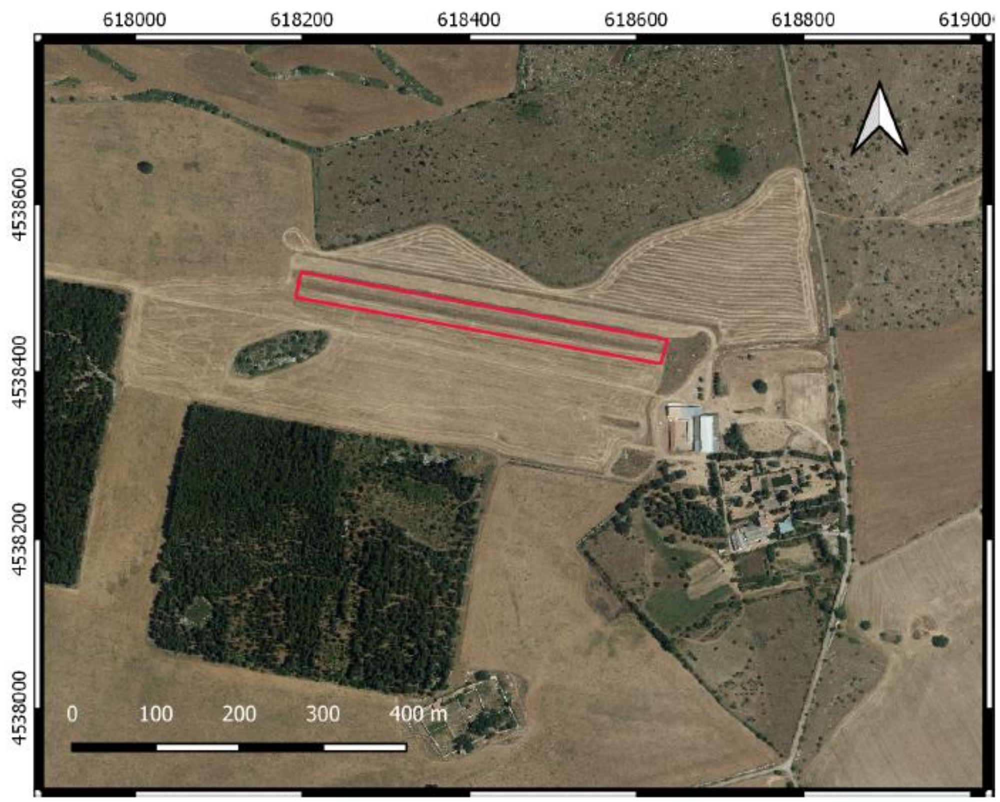

Figure 1 –Restoration test site location on orthophoto
(Source: SIT Puglia – Ortofoto 2019. SR: 32633).

In order to characterize the experimentation areas pedologically, the chemical and physical analyses deemed necessary for these particular kinds of soil were performed, also to evaluate the differences between soils that were exploited in the past using the two different methods mentioned above, as well as to correlate test results with the characteristics of the soil. Thus, two soil profiles were sampled: the former was located in the ploughed land area, and the latter, which was untouched for several years, was located in an open area. This activity was conducted with two repetitions for each soil type, and samples were also extracted for the analysis of the chemical-physical characteristics of the different layers.

The chemical and physical pedological analysis consisted in the following items:

- grain size distribution in soil samples by sieving and/or by sedimentation, including their skeleton;
- determination of organic carbon using the Walkley-Black method and evaluation of organic matter;
- determination of the degree of soil reaction (pH);
- determination of total Limestone and active Calcium Carbonate;
- determination of total Nitrogen, ammonium ions and nitrates, the latter in a KCl solution;
- determination of total Phosphorus and assimilable Phosphorus, the latter with the Olsen Method;
- determination of absorbed Potassium;

- determination of water retention with the Richards Plate method and retention curve with at least two points in addition to those at 0.3 and 15 Bar, both on altered and unaltered soil samples.

Soil profile (Table 1) and values resulting from soil analyses (Tabe 2) are reported below. Between the two areas, main differences in the chemical-physical parameters were in terms of organic carbon, which was higher in the upper soil layer of the unploughed area.

Table 1 – Soil profiles resulting from soil sampling in the restoration test site of Masseria Modesti.

Sample	Francesco Bellino		
Dates	04/03/23 and 30/06/23	**Locality:**	Masseria Modesti
Coord. (utm-wgs84)		**Municipality:**	Ruvo di Puglia
Soil region	72.2	**Province:**	Bari
System	16CA2050		
Stoniness	small = frequent (4-15%) medium = common (2-3%) large = absent		
Rockiness:	absent		
Elevation	500		
Slope (%)	5		
Aspect (°):	S		
Shape	Flat surface		
Substrate	Series of layers and white/grey calcareous banks partially dolomitized, permeable with fissuration and karstification (Calcare di Bari)		
Pedogenetic matter	Local debris; silty		
Use	Not irrigated annual crop		
USDA (10° ed. 2006):	Typic Haploxerolls fine		
Class. WRB:	Limestone Endoleptic Phaeozems		
Layers			
Ap 0 – 20 cm	Moist color 7,5YR 3/2, on small-aggregates; common skeleton (5-15%) of coarse gravel (20-76 mm), irregular shape, calcareous, low halteration (<5%) of coarse gravel (20-76 mm), irregular shape; texture: clay-silty; large polyedric sub-angular structure, moderately developed; highly adhesive; plastic, high water conductivity (1-10 µm/s); fine pores (0,5-1 mm) common (0,5-2%); no effervescence		
Bw 20 - 40	Moist color 7,5YR 3/3, on small-aggregates; no skeleton; texture: clay-silty; large polyedric sub-angular structure, highly developed; very adhesive; plastic, high water conductivity (1-10 µm/s); fine pores (0,5-1 mm), no effervescence		
R	Calcare di Bari		

Table 2 – Chemical and physical parameters tested for each test site in Masseria Modesti.

Parameter	**ploughed**		**unploughed**	
Layer	Ap	Bw	Ap	Bw
Depth cm	0 – 20	20-40	0 – 20	20-40
Skeleton g/kg	182.2	55.6	172.2	55.6

Residual moisture %		4.4	4.6	5.3	4.6
sand g/kg	very coarse	16	9	18	10
	coarse	15	12	19	12
	medium	14	12	22	11
	fine	26	23	25	24
	very fine	39	34	44	36
	total	105	90	128	93
Silt g/kg	coarse	113	105	290	107
	fine	290	254	400	245
	total	403	359	690	352
Clay g/kg		482	551	483	555
pH		8.3	8.2	8.2	8.1
$CaCo_3$ g/kg	total	49.1	21.8	46.7	19.8
	active	30.2	19.5	29.1	12.5
C.O. g/kg		19.7	16.3	22.8	16.6
N t. g/kg		1.69	1.5	1.92	1.6
C/N		11.7	10.8	11.9	10.4
P_2O_5 g/kg		81.8	48.6	96.8	55.6
Condc. µS/cm	Rap. 1/5	305	330	324	330
	P sat.	320	335	320	315
Meq/100g	CSC	31. 7	26.9	32.18	26.54
	Ca	29.1	23	29.5	22.9
	Mg	2.1	2.9	1.7	2
	K	0.4	0.9	0.8	0.9
	Na	0.1	0.1	0.18	0.24
TSB		100	100	100	100
ESP		0.31	0.37	0.5	0.9
Ion exchange	Ca	5834	4233	5429	4215
	Mg	256	353.8	206.8	671
	K	156	351.9	312.8	351
	Na	23	23	41.4	55.2
c.c. g/100 g		35.9	37.4	35.7	36.6
p.a. g/100 g		15.6	16.2	16.7	16.2
Ag/100 gWC		20.3	21.2	19	20.4

Next step consisted of planning tests for the distinct types of pilot interventions for the *restoration* of Alta Murgia’s xeric meadows. In order to conduct different parallel and simultaneous experimental tests, both strips were broken down into an equal number of plots, each measuring 10X20 m. For these smaller plots, various experimental *restoration* techniques were used in order to employ the most effective one in the Murgia environment or, at least, to verify which strategy would eventually be the driver for a positive response.

An herbaceous community of sub-Mediterranean and semi-natural xeric grassland was selected as vegetation reference model to be restored. In fact, this type of grassland is the most typical and representative of the Alta Murgia plateau area and is characterized by a high plant diversity, featuring perennial and annual species. The area is highly valuable in terms of conservation, thanks to its *Acino suaveolentis-Stipetum austroitalicae* association Forte & Terzi, 2005, of the *Hippocrepido glaucae-Stipion austroitalicae* alliance Forte & Terzi, 2005. This type of vegetation is dominated by the endemic species *Stipa austroitalica* Martinovský ssp. *austroitalica*: this entity, which clearly characterizes the physiognomy of the hemicryptophytic grasslands of the Alta Murgia area, is frequently associated with the *Gelasia villosa* (Scop.) Cass. subsp. *columnae* (Guss.) Bartolucci, Galasso & F. Conti, the *Festuca circummediterranea* Patzke and the *Euphorbia japygica* Ten. (Forte et al., 2005).

To identify the different *restoration* techniques to be tested and compared, reference was made to scientific literature and sector manuals (see References). Among the proposed techniques, those considered as most effective for the *restoration* of grassy communities were selected, despite being normally used for restorations in other territorial ecological areas (see *topsoil inversion* or *topsoil removal* – Glen et al., 2017; Kiehl et al., 2010). Mediated techniques from those of literature were also proposed and deemed as more effective for the restoration of the Alta Murgia sub-Mediterranean xeric grasslands.

The testing techniques of the two soil strips (one subject continuously to ploughing after stone removal and the other one left unploughed during the previous 3-4 years except for a minimal periodic mowing) were differentiated, and special attention was paid to the different stage of their natural dynamic process of recolonization. The overall aim, however, is to progressively trigger and/or favour a natural evolutionary process and to allow the topsoil-removed areas to evolve towards selected grassland communities.

Within each strip, hence, tests consisted in applying *restoration* techniques, which were intrinsically different as well as different from those of the other strip, were planned. Three replications were designed for each technique, for a cross-comparison and for a comparison with the control plots, i.e. with those plots where no operations were planned. In addition, among the experimental plots measuring 10X20 m, an interspace measuring 1.5 m was provided to avoid interference during the application of the different processes. In other words, the creation of 21 experimental plots was designed for the application of 6 different *restoration* techniques that were replicated in 3 different plots. The three remaining plots where no operations were planned had to function as experimental controls and act as a test themselves, to evaluate the recolonization natural process of spontaneous vegetation.

The experimental scientific project was implemented involving the application of the *restoration* techniques summarized below:

- Ploughed strip

A. control plot (ploughed land);

B. *topsoil inversion* and soil recompression with rolling;

C. *topsoil inversion*, propagule sowing and soil recompression with rolling;

D. *topsoil inversion*, spreading of chopped hay, compression by rolling and covering with jute bionet;

E. harrowing and rolling compression;

F. harrowing, propagule sowing and rolling compression;

G. harrowing, spreading of chopped hay, compression by rolling and covering with jute bionet;

- Fallow strip

H. control plot;

I. light harrowing, propagule sowing and compression by rolling;

J. light harrowing, spreading of chopped hay, compression by rolling and covering with jute bionet;

K. light harrowing and seed sowing of chamephytes from the Alta Murgia pastures (e.g. *Euphorbia spinosa*, *Euphorbia japygica*, *Euphorbia myrsinites*, *Satureja montana*, *Ruta graveolens*, *Rhamnus saxatilis*);

L. *sod cutting* or *turf stripping*, propagule sowing and light compression by rolling;

M. *sod cutting* or *turf stripping*, spreading of chopped hay, light compression by rolling and covering with jute bionet;

N. *sod cutting* or *turf stripping* and sowing of chamephytes from the Alta Murgia pastures (e.g. *Euphorbia spinosa, Euphorbia japygica, Euphorbia myrsinites, Satureja montana, Ruta graveolens, Rhamnus saxatilis*).

The developed experimental design is shown in the following figure and was created according to a randomized block scheme.

Striscia arata

Repliche	Trattamento
1	D
1	A
1	E
1	C
1	F
1	B
1	G
2	B
2	A
2	F
2	C
2	G
2	D
2	E
3	B
3	D
3	F
3	C
3	A
3	G
3	E

Striscia soda

Repliche	Trattamento
1	H
1	L
1	N
1	K
1	J
1	M
1	I
2	L
2	M
2	I
2	H
2	K
2	N
2	J
3	I
3	H
3	L
3	K
3	J
3	M
3	N

Figure 2 – Experimental design of *restoration* techniques. Ploughed and unploughed strip on the left and right, respectively. Explanations of symbols in the text.

After selecting the *restoration* techniques, the overall implementation of plant propagation material was defined keeping in mind one of the key principles of Restoration Ecology, namely that of not just using propagation indigenous material but also material deriving from local plant populations (Di Sacco et al., 2021).

Consequently, the methods, locations, supply times and necessary quantities of plant material were also identified, the latter mainly consisting of chopped hay and flower sowing to be used in the different experimental plots. Hay was collected through harvesting (mowing) at the beginning of summer in a purposely identified area (source site) in Jazzo Coppa di Sotto (Fig. 3). Despite being characterized by perennial grassland not attributable to the Habitat *sensu* Directive 92/43/EEC, this location hosts a large part of species populating the selected grassland used as a reference model. This solution was adopted to avoid a negative impact on EU Directive Habitats. In addition, the vast majority of the Alta Murgia protected grasslands are made up of xeric and sub-Mediterranean herbaceous communities, often showing a strong presence of *Stipa austroitalica*: the latter, being a species included as a priority in Annex II of Directive 92/43/EEC cannot be collected, unless a derogative authorization is granted by the Ministry of the Environment. Furthermore, this technical solution was implemented to avoid commercial mixtures, which would be uncapable of contrasting floristic pollution (i.e. with species foreign to the Alta Murgia environment) and genetic pollution (i.e. non-native propagation material - thus, not originating from local populations).

The mowing-derived material was partially employed to obtain shredded hay that was destined to 4 experimental plots, for 3 replications each - i.e. in 12 plots for a total surface area of 2400 m². The quantity of chopped hay was approximately 100 -140 kg per plot. For the remaining hay, manual threshing was disposed in order to free the propagule sowing which would then be used in 4 experimental plots for 3 replications each - i.e. also in this case, in 12 plots with a total surface area of 2400 m². The quantity of required flower sowing was approximately 3.0-3.5 kg per plot.

As for composition, structure, biomass production and seed content of the estimated herbaceous vegetation, a mown surface area was calculated in the donor site equalling to approximately 3.5 - 4 ha.

The overall calculation of the required quantities was based on the data deriving from the sector manuals (see Reference), also in regard to the floristic and ecological peculiarities of the Alta Murgia grasslands.

Figure 3 – Location of the donor site (source site) for mowing on orthophotos (Source: SIT Puglia – Ortofoto 2019. SR: 32633).

We opted for a manual sampling of the chamephytic species seeds required for the *restoration* operations, so that no vegetative portions of the mother plants would suffer any cutting. Suitable areas for the collection of the seeds were localized the basis of territory knowledge as well as taking reference from recent literature data (Tomaselli et al., 2021) (Fig. 4). The seeds were used, as indicated above, in 2 experimental plots for 3 replicas each, i.e. in 6 plots with a total surface area of 1200 m². The hypothesized seed amount is 200 seeds per m², equal to approximately 70-200 grams per experimental plot, depending on the prevalence of species presenting either light seeds (e.g. *Satureja montana* – weight of 1000 seeds: 0.366 grams) or heavy ones (e.g. *Rhamnus saxatilis* – weight of 1000 seeds: 10.08 grams).

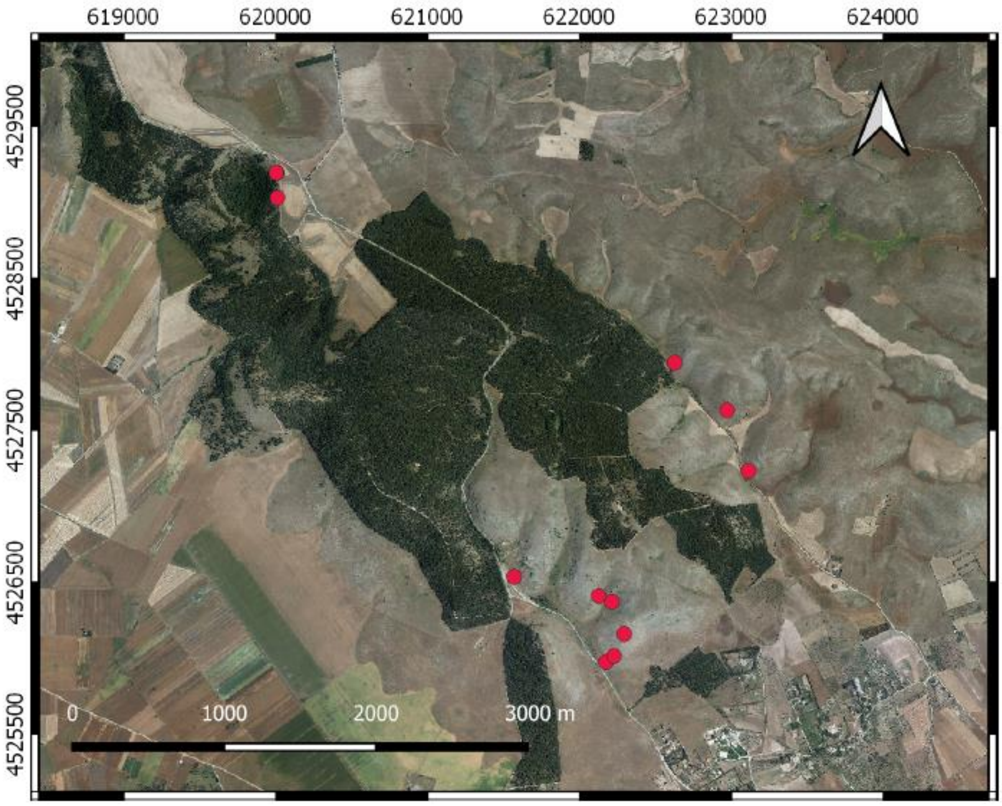

Figure 4 – Location of the germplasm sampling stations of the chamephytic species on orthophotos. (Source: SIT Puglia – Ortofoto 2019. SR: 32633).

After the *restoration* testing planning phase, activities included the drafting of the Environmental Impact Assessment (VINCA) document. This was a necessary step, since both the testing areas and the supply of vegetal material fall within the SAC/SPA Murgia Alta. In addition, scientific technical support was provided to the company that carried out the pilot interventions for all the cultivation procedures, from the collection of the plant material made up of hay, to its processing and storage and its planting in the various experimental plots. Furthermore, support was also provided for selecting the bionet to be used and the tools to be used for the different crop operations, with regular management and in-field meetings during the various phases of this study.

Unlike hay, the collection of implant vegetal material consisting of the germplasm of the chamephytic species was disposed by the Department personnel. The company involved in other cultivation phases was not employed for this instance, as this phase required the expertise of trained and specialized collectors: in fact, operators must be capable of dealing with tasks like identifying mother plants, phenological monitoring, as well as collecting, cleaning, and preparing the propagation material.

Among all the chamephytic entities identified in the planning phase, it was only possible to collect the germplasm of those which ripened a good quantity of fruits and seeds in the harvest year 2022. The phenological monitoring phase in the occurring sites of these species is generally required in order to select an optimal harvesting time. During this phase, suitable plants identified for a possible germplasm collection only belonged to the species *Euphorbia japygica, Rhamnus saxatilis, Ruta graveolens* and *Satureja montana*.

The sampling of fruits and seeds according to species occurred between August and November 2022, and was preceded by running a *cut-test* directly in-field to determine the viability and maturation of the germplasm to be collected. The collected material was placed in paper bags and taken to the laboratory for the subsequent processing phases. Then, the different fruits and/or seeds were placed in open plastic containers and placed in rooms for post-ripening and quarantine. Subsequently, the germplasm of each species was cleaned and selected as specified below.

For *Euphorbia japygica*, the capsules were stored inside organza fabric in order to prevent any seed spillage outside the container. Subsequently, the separation of the seeds from the rest of the organic material was carried out using screeners with meshes of different sizes. Finally, seeds were sorted manually using a binocular stereoscopic microscope. The seed lot was then characterized by determining the weight of 1000 seeds and their viability, with the *cut test* method. The entire lot was made up of approximately 12,600 useful seeds for translocation.

In the case of *Rhamnus saxatilis*, the collected nuculas (stone fruits, typical of this entity), were stripped of their pulp, and the seeds obtained were firstly washed in running water and then left to dehydrate under controlled temperature and humidity. Subsequently, seeds were selected under a binocular stereoscopic microscope. The batch was then characterized, by determining the weight of the 1000 seeds, thus allowing an estimate of its numerical consistency. Vitality was also assessed with the cut test method. The seeds available for the subsequent translocation procedures amounted to 23,400.

For *Ruta graveolens*, the seeds were cleaned and initially selected using screeners with meshes of various sizes. Then, a second selection was applied using, also in this case, a binocular stereoscopic microscope. The lot was characterized determining the weight of the 1000 seeds and, after weighing the entire lot, the numerical consistency resulted in 39,000 seeds. Vitality was also assessed for this batch, with the *cut test* method.

Finally, for *Satureja montana* the seeds were cleaned and selected, using the same procedures as for the previous species (i.e. cleaning and selection by sieving and using a binocular stereoscopic microscope). The characterization through the weight of 1000 seeds enhanced an evaluation of the actual, numerical consistency of the lot, which equalled to 51,000 seeds. Vitality was also assessed for this batch with the *cut test* method.

Eventually, all the available seeds were divided into six lots, one for each respective *restoration* plot. Each batch of seeds consisted of 21,000 seeds, divided as follows: 2,100 seeds of *Euphorbia japygica*, 3,900 seeds of *Rhamnus saxatilis*, 6,500 seeds of *Ruta graveolens* and 8,500 seeds of *Satureja montana*.

With regard to the restoration experiment, which has been concluded in winter 2022, it is not currently possible to verify the effectiveness of the method and provide indication regarding the best methodological option on the basis of the results. As expected, due to natural vegetation dynamics, it is not possible to assess the differences among different restoration actions. This result at the early stage after the experiment is not surprising, because the establishment of the selected sub-Mediterranean communities need more than one season, and is anticipated by early short-lived colonizer plants, i.e. nitrophilic, ruderal and synantropic species. An accurate assessment of the ongoing dynamics, in turn providing quantitative and qualitative indications of the effectiveness of restoration practices, will be carried out during 2024 and subsequent years. However, significant results may be obtained not earlier than the end of the third or fourth year after the experiment.

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