## Allegato 4a\_6

# A protocol to monitor the phenology and nutritional content of grasslands

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#### Introduction

Studying the changes in phenology of vegetal communities is fundamental for the knowledge and the conservation of the populations which live into those habitats. In particular, in Gran Paradiso National Park the beginning of alpine grasslands growing season have been anticipating during the last 30 years, and in particular between 1990 and 2000 (see action 4.a.2). This change can be linked with the reduction of Alpine ibex population (*Capra ibex*), which drastically reduced from 1992 to 2008 (Mignatti et al. 2012; Pettorelli et al. 2007).

Continuing to study grassland phenology in the future will be important to better understand this link. To do it, prediction of nutritional content from remotely sensed data can be an easy way to obtain this information; however, results of model validations have shown that their strength to different climatic situations need to be tested using data of different seasons (with the exception of biomass, which seems quite strength to these variations). So, other field data collections appear to be useful for two reason: not only for the continuous improvement of the built models, but also to obtain sample data of real nutritional and phenological contents.

For this purpose, a possible protocol for performing this phenological and nutritional grassland monitoring have be shown in this document.

#### **Field methods**

During 2012 growing season, data were collected within 19 experimental plots (see figure 1). These plot have been chosen within alpine grasslands taking into account these restrictions:

• a minimum distance of 500 m from the woodland, to avoid the possible interaction of this surface in the determination of the NDVI pixel value (potentially very dangerous using composite data, as the MOD09Q1 are);



Figure 1: Maps of the 19 experimental plots and table of each position and elevation. Coordinates are in UTM projection (32N), datum WGS84; units are in meters. Grassland surface (taken from the land use map of the Gran Paradiso National Park) are marked with green polygons.

- a minimum distance of 1 kilometre between plots, to minimise the autocorrelation between MODIS values;
- a coverage of the altitudinal range of the grasslands and of the different exposures.

Each plot is composed of a squared surface of  $3 \times 3$  m, enclosed to prevent grazing from domestic and wild herbivores. Enclosures consist of an internal protection with fence (to avoid ungulate grazing) and a wire mesh (to take marmots out); in case of presence of domestic ungulates, an additional external fence have been placed (see figure 2).

During 2013, phyto-pastoral analyses have been performed on the 19 experimetnal plot (for details of the methods, see action 4.a.3).

The enclosures have been located on an homogeneous surface, enough representative of the surrounding buffer of 300 metres (in terms of exposure, slope and microhabitat). Each plot surface has been divided into 15 sectors of  $50 \times 50$  cm; during each sampling, we perform a cutting of all the grass present in one of these sectors (with the exception of the eventual dry grass remained from the year before). Also, a  $1 \times 1$  m surface has been reserved to take, each time, measures about grass height



Figure 2: Example of experimental enclosure: external view (with the two protection levels), internal view (cutting areas on the left, measuring area on the right) and a detail of the measure area with the grid used to take measures (yellow circles represent the 16 measured squared).

(taking 16 measures into each square).

When possible, this measures and cuttings have been repeated also out of the enclosure, to test the difference between grazed and not grazed grass.

Data collection has been done from the beginning of the growing season (second half of May – end of June, depending on the plot) to the end of September (when all the plot communities reached the senescence), with an interval between samples of two weeks. With this experimental design we obtained, for each plot, from 4 to 10 samples, for a total of 142 records.

Collected samples have been weighted (wet weight), dried in a ventilated oven at 60°C for 48 hours and weighted again (dry weight). Bromatologic analysis have been performed in winter 2012-2013, to obtain, for each sample, relative contents of crude protein (AOAC 1990), neutral detergent fiber (Mertens et al. 2002), acid detergent fiber and lignin (AOAC 2000), digestibility after 24 and 240 hours (Goering and Van Soest 1970). To perform digestibility analyses, samples are subjected to the action of digestive rumen bacteria for 24 or 240 consecutive hours, keeping their physiological conditions of the rumen. At the end of digestion the proportion digested NDF has been estimated. This determination is needed to identify the nutritional goodness of the forage analysed. In the case of 240 h digestibility, the final estimation is actually the indigestible proportion, not able to provide useful energy to the rumen.

These data have been used also to produce a calibration curve for the NIRS system, which will allow to have data at low cost and in a short time for the future samples.

#### Results

Figure 3 represents the seasonal trend of each variable (series taken into the enclosures are generally more complete because their cutting has been considered prior). While protein content and digestibility



Figure 3: Seasonal series of nutritional measured variables into each plot: points marked with + refer to the samples cut into the enclosure, the ones with  $\times$  outside it. Dotted lines represent predicted values (see action 4.A.2).

test values and paired Student's t test<br/>values between inside and outside en-<br/>closure measures.Variable  $\rho$  t  $\mathbf{p}(\mathbf{t})$ Biomass0.50-1.220.23Crude protein0.80-0.930.36

Lignin

0.66

0.70

0.67

0.76

0.60

0.68

-3.24

-2.25

-0.91

-1.65

-1.46

1.83

0.0018

0.028

0.072

0.36

0.10

0.15

Neutral detergent fiber

Acid detergent fiber

Digestibility at 24 h

Digestibility at 240 h

Available protein

Table 1: Pairwise Spearman's rank correlation  $\rho$ 



Figure 4: Comparison between biomass and mean grass heights of inside-enclosures samples. Heights are log-transformed to avoid heteroscedasticity.

at 24 hours present a clear decreasing trend, fiber increases during the season. Biomass, available protein and digestibility at 240 hours do not show a clear trend (but it is possible to notice an increase of biomass and available protein in the first part of the season, and a decrease of available protein at the end).

The main difference between inside-enclosure and outside-enclosure measures appear to be that the residual variance of the seconds is higher: that can be attributed to the disturbances which can be present between each cutting (particularly where domestic animals are present). However, generally the two series does not differ so much. Table 1 shows the values of pairwise Spearman's rank correlation  $\rho$  tests: it is possible to notice that almost all the variables presents  $\rho$  values greater than 0.60.

We analysed also the presence of a significant inside-outside shift between each variable using paired Student's t tests (see table 1): generally there are no difference (only neutral and acid detergent fiber differ significantly, with a higher fiber content into the outside-enclosures samples).

A pairwise Spearman's rank correlation  $\rho$  test has also been performed between grass heights and biomass measures:  $\rho = 0.75$ . Computing a univariate linear model between this two variables (logtransforming heights to avoid heteroscedasticity problems) we obtain an adjusted R<sup>2</sup> value of 0.57.

### **Discussion and conclusions**

We tested a method to take field measures about nutritional content of grassland, with the objective to link it to remotely sent information. This method in general resulted valid also as a standalone protocol to monitor the evolution of nutritional content of grassland during time. Field data collection in the future will be important also considering the conclusions of action 4.A.4, since the strength of the remote models with different climatic situations need to be verified.

For these reasons, here we proposed a fast method useful in the future to monitor nutritional changes into alpine grasslands.

**Experimental plots** To perform a temporal monitoring which can show temporal changes, we suggest to maintain the same plots into the time instead of changing them randomly (in this case, plot number should be very high). We indicate five possible plots, chosen for its homogeneity (useful to link them to remote data), for the absence of domestic disturbance and to cover the vegetation heterogeneity. We avoid the highest plots (2600-2800 m) because the short season and the low nutritional importance make difficult their monitoring.

- Casotto della Vaudalettaz (same position of plot VAU-CAS), classified as Festuca gr. ovina type (facies 19.22 Mesofila oligotrofica ad Anthoxanthum alpinum e Festuca gr. ovina). Meadow is not very large, but sufficiently to take remote data; it looks quite homoegenous, and it will be useful to test the evolution of a grazed grass which is not grazed since some years. Monitoring would be facilitated by the presence of a guard hut.
- *Plan de feye* (position 354756 E, 5045916 N, UTM32N WGS84): this plot was not monitored, but its position look preferable than the enar used one (ENT-VAL) due to the absence of domestic animals. In addition, 3 years ago a mudslide interested that area, which now is quite fragmented.
- Vallone di Levionaz (same position of LEV-MEZ, or, a bit better, near 362648 E, 5048279 N, UTM32N WGS84), classified as Sesleria varia type (facies 13.06 Mesoxerofila a Sesleria varia). The importance of this meadow is due to the presence of one of the biggest ibex population of the Park. Monitoring would be facilitated by the presence of a guard hut and of a research group.
- Piana del Nivolet, (position 355826 E, 5039480 N or 356319 E 5040027 N, UTM32N WGS84): Nivolet is a heterogeneous and disturbed grassland (high presence of tourists, some – not many – beefs and sheets in the end of the season); however it is a very large grassland, so a plot appear necessary. The shift of plot position is due to the fact than NIV-SBA was very poor in terms of biomass, and NIV-ALP was too much heterogeneous.

• Alpe Fumetta (same position of ORC-FUM), classified as Carex sempervirens type (facies 32.XX Mesofila mesotrofica a Crex sempervirens, Trisetum flavescens, Trifolium pratense). As VAU-CAS, meadow is not so but sufficiently large, homogeneity is good, there are no domestic animals and the location is owned by the Park.

**Enclosures** Enclosures have shown to decrease residual variance, but not to cause significant shift within nutritional main components. Furthermore, they should be assembled and disassembled every year, because winter snow would damage them. A more robust structure does not appear adequate, since maintaining the same exact position every year could induce alterations into herbaceous communities. In addition, enclosed plots should be visited regularly to prevent damages. For these reasons, for a monitoring the presence of enclosures does not appear necessary. To reduce residual variance, we suggest to cut more than one sample for each plot (see paragraph below).

**Temporal frequency** The higher it is, the more reliable seasonal series are. As a mediation, we propose a lower frequency of cuttings (since nutritional component appear more stable) and an higher frequency of height measures (since monitoring phenology require more measures, above all in the first part of the season). So, we propose four monthly cuttings and biweekly height measures (weekly in the first month from beginning of growing season). Grass height measure will be used also to estimate biomass, since a correlation between the two measures has been shown.

Field work The framework shown in section is proposed, with these differences:

- cutting 8  $25 \times 25$  cm squared areas instead of one  $50 \times 50$  cm, whose position will be randomly chosen in the offing of the plot;
- taking height measures in the same position (which have to be permanently marked on the ground), even without enclosures.

**Laboratory analysis** Nutritional components will be obtained using NIRS curves obtained from 2012 samples, so analyses will be faster and cheaper.

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