BioPast – An Integrated Approach to
Sown Biodiverse Permanent Pastures Rich in Legumes
(PTDC/AGR-AAM/69637/2006)
Final Scientific Report

Aims of the project

The aims of the project were:

- Determine the relationships between soil properties, pasture productivity, botanical composition and animal performance in sown biodiverse permanent pastures rich in legumes (SBPPRL) and establish the setup for their long term monitoring at Quinta da França;
- Contribute to the establishment of long term organic matter measurements for SBPPRL;
- Determine the persistence of the set of species originally sown in SBPPRLs, and their relevance for animal production;
- Optimise the management of SBPPRL for carbon sequestration;
- Determine the existence of nitrogen leaching risks in SBPPRLs;
- Model the SBPPRL system;
- Analyse the economic and environmental impacts of the large scale implementation of SBPPRLs;
- Establish pasture management recommendations for the participating farmers, through the Extensity network.

Brief description of activities developed and changes occurred in the execution of the project

During the project an extensive characterization of the SBPPRL system was done, by collecting and analysing field data. The activities aimed at gathering information to allow a comparison between SBPPRL and natural grasslands and identifying the advantages and disadvantages of the SBPPRL system.

In the field, there was an intensive work done in the Quinta da França farm. The work included monitoring of soil organic matter (SOM) and other soil parameters, at different depths and soil characteristics. The analyses were done both in the SBPPRL system and in natural grasslands. The goal was the comparison of the parameters gathered between the two systems.

The activities included an analysis of the N mineral dynamics, namely to understand if the accumulation of N entails phenomena of leaching and denitrification. The analysis of organic N was also included in order to understand if SBPPRL allow a higher concentration of N in the soil than natural grasslands. In order to understand the effect variables such as wet versus dry years and soil depth, passive variables in indirect

ordinations were used, adding the cover of the characteristic species of precise phytosociological classes. In order to understand the effect of species diversity on ecosystem function an analysis was made of the influence of SBPPRL on the slope, microenvironmental spatial arrays and climate fluctuations as well as on soil gradients. At this farm we also studied the dynamics of cattle and sheep flocks.

Besides Quinta da França, data was gathered in another 11 farms. The data gathered, along with that gathered in Quinta da França was used in the integrated assessment of SBPPRL described below.

We developed a theoretical model of soil organic matter, which was calibrated with the field data. The main goal was still the comparison of SBPPRL with natural grasslands. Since an increase in soil organic matter is directly related to an increase in carbon, it is possible to estimate the carbon sequestration. In order to perform a carbon balance it is necessary to include all sources and sinks (animals, liming, legumes). A Life Cycle Approach was also developed to analyse the direct and indirect environmental impacts of SBPPRL compared to natural grasslands. In a more qualitative perspective, we also performed an integrated analysis of the consequences of the implantation of SBPPRL.

In July 2010 the partners asked FCT for an extension of the project deadline (2010/07/31) to 2011/01/31. This request was based on the following reasons:

- Data collection in 2008/2009 was not successful due to observed weather conditions (lack of rain, leading to insufficient pasture growth). Since two years of field data were foreseen, data collection had to be extended to the 2009/2010 farming year.
- To allow the publication, during the project, of enough papers and presentations to fulfill, in a general manner, the publication goals of the project.

FCT replied affirmatively to this request. In March 2011 the partners asked FCT for another extension of the project (until 2011/07/31). This was in order for a better fulfillment of the publication goals of the project (giving the project more time for the publication of papers who had been submitted) and also to complete lab work on the nutritional quality of pastures. FCT's response was negative.

Aims Reached

With the work developed during the project it was possible to prove that, in comparison with natural grasslands, SBPPRL show high benefits, regarding productivity and therefore stocking rate, soil quality, adaptation to field and climate constraints and carbon sequestration. The possible "N problem" was also proven not to be an issue. These topics are developed below.

The data gathered in Quinta da França allowed us to study the role of N dynamics in SBPPRL as well as the effect of SBPPRL biodiversity on soil and climate conditions and the flock dynamics

The role of mineral N in leaching and denitrification was assessed. Two experimental strategies were adopted in a stabilized SBPPRL in Quinta da França. The results showed that N mineral availability in the soil was always very low. Consequently, the risks of

leaching and denitrification were also low. Regarding organic N, it was concluded that in SBPPRL the SOM C/N ratio is decreasing at the same time as the potentially available N is increasing. This trend has a positive feedback on soil fertility, pasture biomass yield potential and, finally, on animal biomass output.

Considering the study of the effect of SBPPRL biodiversity on soil and climate conditions, results confirmed the hypothesis that diversifying the sown species/cultivar colonists' pool in seed pasture mixtures appears to make the pastures more resilient to interannual climate and soil gradient fluctuations. Soil heterogeneity tracking was indeed translated into higher productions at the pasture scale, and in a buffering of dry matter (DM) production along soil catenas. (i.e., SBPPRL do counterbalance soil gradients). This is indirect evidence that interannual yield variations in SBPPRL are smaller than in semi-natural pastures.

The use of passive variables in indirect ordinations, namely the % area cover of the characteristic species of precise phytosociological classes allowed the tracking of the effect of wet vs. dry years or of soil depth (Aguiar *et al*., 2011c; Aguiar *et al.*, in prep.). These approaches, widely used in vegetation science, could be generalized to other soil proprieties besides soil depth in order to gather indirect, semi-quantitative, information about soil conditions from species abundance that would be useful in day-to-day SBPPRL management.

In terms of animal performance, two main achievements must be noticed. First is that the gathered data did not allow a correlation between annual and inter-annual flock distribution and environmental variables or species/varieties spatial distributions. The second is that the genotyping methodology to simultaneously genotype eighteen SNPs (Single Nucleotide Polymorphisms) in S1-casein (CSN1S1), S2-casein (CSN1S2), -casein (CSN2), -casein (CSN3), -lactoglobolin (LGB) and prolactin (PRL) genes in sheep was successfully established.

The data gathered in Quinta da França, along with the data of the other 11 farms, made it possible to understand the role of SOM in SBPPRL, namely that SOM reaches higher concentrations in SBPPRL compared to natural grasslands. It was also possible to prove that SBPPRL are more productive than natural grasslands.

The model developed showed that the expected steady-state long term SOM concentration in undisturbed SBPPRL is higher than in natural grasslands. The results indicate that both the first-year dummy for SBPPRL, and the dummy for NG were not significant. We started by removing the least significant one, the first-year dummy for SBPPRL and then the dummy for NG. The new results also show no significance for the NG dummy. This means that fertilized plots do not increase their SOM pool significantly more than non-fertilized plots. In 10 years, there is an average increase of 0.19-0.20 percentage points per year in SBPPRL (equivalent to 1.33-1.40 t C.ha-1.yr-1 or 1.48-1.56 t C.ha-1.yr-1, depending on the soil density), against an increase of 0.13-0.14 (0.94-0.95 t C.ha-1.yr-1 or 1.05-1.07 t C.ha-1.yr-1) for fertilized natural grasslands and 0.07-0.08 (0.51-0.53 t C.ha-1.yr-1 or 0.57-0.59 t C.ha-1.yr-1) for natural grasslands. This SOM represents an increase in carbon, and even when the animal, legume and liming emissions are considered, the balance is still favorable to SBPPRL.

In a more integrated approach, SBPPRL is associated to a higher environmental and social value. Increasing SOM also improves soil nutrient availability and therefore plant productivity, which allow a higher stocking rate. The higher stocking rate represents an advantage in terms of economic viability, since, for the same area it is possible to raise more animals, but also because the need for concentrated feeds decreases. Since the pastures are actively grazed, there is no invasion by shrubs, which represents an environmental advantage.

We used a Life Cycle Approach to quantify the direct and indirect impacts of SBPPRL compared to natural grasslands. Since SBPPRL are more intensive in terms of inputs (namely fertilizers), an analysis per hectare, not accounting for the animal production, shows that the SBPPRL are only favorable regarding the greenhouse gases emission. However, SBPPRL are much more productive and allow a reduction in the use of concentrated feed in animal production. When the assessment takes this into account, the SBPPRL are favourable for every impact category analysed (greenhouse gas emisions, ozone layer, acidification, eutrophication, heavy metals, carcinogens, winter smog, summer smog and energy resources).

BioPast has provided scientific knowledge on carbon sequestration at pastures that has been supporting policies, discussion groups and government funded projects under the framework of Portuguese commitment to Article 3.4 of Kyoto Protocol. More specifically, the research on the dynamic of soil organic matter at biodiverse pastures has allowed the estimation of carbon sequestration factors that have been made public in a paper published at Ecological Modelling. This paper became a reference both to the Portuguese Carbon Fund (FPC) and to the National System for the Estimation of Emissions by Sources and Removals by Sinks of Air Pollutants (SNIERPA).

FPC has been funding a project that remunerates carbon sequestration to farmers that install biodiverse pastures and follow specific management practices. So far, around 500 farmers have been remunerated by this environmental service provided at 27 000 ha. Another funded project is just starting, which will expand this concept of environmental services valuation to 30 000 ha more. This is all additional carbon sequestration that Portugal has to report at its National Inventory Report (NIR) of anthropogenic emissions by sources and removals by sinks of greenhouse gases, as an Annex I Party to the Convention on Climate Change. The use of the above mentioned carbon sequestration factors is part of the methodology that has been approved by the Working Group of SNIERPA on Article 3.4 to estimate both the additional and non-additional carbon sequestration at grasslands for NIR.

The broad communication of these results occurred at international summits such as the 12th Meeting of the FAO-CIHEAM Sub-network on Mediterranean Pastures and Fodder Crops (Elvas/Portugal, 2008), the United Nations Climate Change Conference (Copenhagen, 2009) and the Expert Group on Agriculture and Climate Change 1st Meeting (Brussels, 2010). The BioPast project also raised the attention of an Australian technician that used them to support the recommendation of a similar approach to the Australian Government.

The project was awarded three prizes:

- The "Prémio Progresso dos Pastos", awarded by the Portuguese Society of Pastures and Forage, was granted to posters produced by the project in the 2008 and 2009 editions; the posters were, respectively, Teixeira *et al*., 2008d and Castro and Castro, 2009;
- Ricardo Teixeira, the main author of one of the referred posters, was given an honorary mention by the Portuguese Order of Engineers (South) in the "Prémio Inovação Jovem Engenheiro 2007" by his work on the subject of the project.

Financial execution and brief justification of deviations from the budget

The financial execution of the project (assuming that the last expenses submitted are approved by FCT) was over 90%. Instituto Politécnico de Bragança did not spend €2.407 and spent €474,21 in excess on Missions. This is due to the extension of the project for a year after the foreseen end date, that led to more travel to perform fieldwork and present project results in science meetings. The project coordinator will ask FCT for the transfer of this amount from IPB's "Services" to IPB's "Missions". There were no significant budget transfers between expense categories or between partners. The greatest transfer was of about €8.000 to Services to pay for fieldwork that was originally thought to be paid by scholarships.

Task 1 – Soil and Vegetation Ecology

Researchers Involved

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Results

The expected results in this task were:

- Build a longer data series of information on an SBPPRL sown in 2000 in Quinta da França and monitored in the period 2000-03, in the following characteristics: biomass production; botanical composition; nutritional quality; soil fertility; stocking rate;
- Evaluation of N fixation and C sequestration in stable soil organic matter by SBPPRLs;
- Evaluation of inorganic N leaching risks in Autumn-Winter;
- Evaluation of N fixation in stable soil organic matter by SBPPRLs;
- Evaluation of the dynamics of nutrient release by the aerial phytomass;
- Evaluation of the effect of soil heterogeneity and local pasture composition on these processes;

- Evaluation of the effect of SBPPRL on some chemical and physical soil proprieties;
- Explore the utility of species/varieties as bioindicators of soil proprieties.

A conceptual model of carbon (C) and nitrogen (N) cycle processes contributing to the GHG balance in grasslands can be read as follows. Legumes and grasses grow in consociation using atmospheric CO_2. Symbiotic associations of legumes and microorganisms, namely Rhizobium fix atmospheric nitrogen as well. Belowground, a complex set of reactions between plant (roots), soil mineral particles, microorganisms and macrofauna (earthworms, etc.) takes place (Teixeira, 2010). Three important outputs, in terms of GHG come out of the sum of these processes: SOM accumulation in soils, leaching of SOM particles and dissolved N, and emission of N_2O to the atmosphere. The aboveground part of the plants is eaten (on site or off-site) by animals, and be complemented by feeds (C and N import). Either through the process of enteric fermentation or from the degradation of wastes, there is some emission of CH_4 and N_2O. Finally, there is application of limestone to increase pH, and there is a corresponding emission of CO_2 (Teixeira, 2010). This subject is further analysed in tasks 4 and 6.

SBPPRL are dominated by annual legumes, namely T. subterraneum. Legume dominance can surpass 50% cover in SBPPRL sown with a correctly chosen seed mixture in soils with a properly managed chemical fertility and intensively grazed all year by cattle and/or sheep (Aguiar *et al.*, 2011d). On the other hand, SBPPRL are more productive (Teixeira *et al.*, 2010) and show evidence of more intense microbiological activity (Pereira *et al.*, 2010a) than semi-natural pastures. The productivity and the dominance of annual legumes in the SBPPRL raise an important question: in any moment of the biological cycle of SBPPRL annual species, can peaks of N mineral availability in soil (which can be lost by leaching or denitrification) occur? This risk could be significant after the first autumn rains, when intense microbiological activity and severe SOM (soil organic matter) mineralization are expected, while the annual sown species display a low nutrient uptake capacity due to an exiguous root biomass and soil restrictions to oxygen diffusion (this risk was reinforced by the empirical data gathered in Biopast that revealed that compaction in SBPPRL soils in autumn can be very high, imposing severe limitations to root growth and to oxygen diffusion). Leaching and denitrification risks in autumn are almost exclusive to Mediterranean annual pastures rich in legumes because temperate pastures exhibit smaller legume covers and are dominated by perennial plants (usually grasses) with a root system soon active after the Autumn rains or that does not suspend its activity in summer.

To track the risks of leaching and denitrification, two experimental strategies were adopted: 1) a discontinuous approach using soil samplings at regular time intervals, and 2) a continuous approach using nitrophilous species with an early season mineral N demand (*Brassica napus*) or a late season mineral N demand (*Secale cereale*). Both were used in a stabilized SBPPRL located in Quinta da França (Portugal). The results showed that N mineral availability in the soil was always very low. Consequently, the risks of leaching and denitrification were also low (Rodrigues *et al.*, 2010b). These conclusions are fundamental for an accurate environmental assessment of SBPPRL.

Beside the N mineral pool, the N organic pool was also studied. Both pools (mineral and organic soil N) are indispensable in an evaluation of N dynamics in SBPPRL. Being highly productive, it is expectable that SBPPRL accumulate an organic N pool in soil more quickly than semi-natural pastures. SBPPRL's high legume content should also favor a lower C/N ratio in SOM. In a comparative study, soil cores were sampled in an SBPPRL and in a control semi-natural pasture with a very low legume cover and a low productivity, located in the same soil catena (similar ecological conditions) of the SBPPRL. Both were located in Quinta da França (Portugal). Soil organic N was evaluated in the soil cores by the Kjeldal method and the potential available N (sequestered in SOM) was determined in pot experiments with Italian rye-grass. It was concluded that in the SBPPRL the SOM C/N ratio is decreasing at the same time as the potentially available N is raising. These trends are probably due to a progressive enhancement of the organic debris input in the SBPPRL soil and its more favorable C/N ratio (Rodrigues *et al*., 2010c). This trend has a positive feedback on soil fertility, pasture biomass yield potential and, finally, in animal biomass output.

The sown pastures literature is focused on agronomic management and biomass yields. Ecological studies at the community scale relating soil proprieties with pasture composition in Mediterranean environments are almost restricted to natural and semi-natural pastures. On the other hand, although an accurate measure of the realized niches of Mediterranean plants (like the Ellenberg's indicator values developed for central Europe) is not yet available, the Iberian phytosociological bibliography could provide a powerful ecological classification of indigenous plants that could be generalized to sown pasture ecology studies.

We were able to prove the utility of the community scale approach and of the "phytosociological optimum" concept in the sown Mediterranean pastures ecology study (Aguiar *et al*., 2011c; Aguiar *et al*., in prep.). The use of passive variables in indirect ordinations (e.g., PCA – Principal Components Analysis) adding the cover of the characteristic species of precise phytosociological classes allowed us to track the effect of wet *vs.* dry years (cover of *Isoeto-Nanojuncetea* characteristic species) or of soil depth (cover of *Molinio-Arrhenatheretea* and *Helianthemetea* characteristic species) (Aguiar *et al*., 2011c; Aguiar *et al*., in prep.). These approaches, widely used in vegetation science, could be generalized to other soil proprieties besides soil depth in order to gather indirect, semi-quantitative, information about soil conditions (e.g. available P and pH) from species abundance that would be useful in day-to-day SBPPRL management.

In the SBPPRL, sown species/cultivars should provide less ecological information than indigenous species, for two mains reasons. First, the number of improved sown species/cultivars in SBPPRL mixtures is limited. SBPPRL usually include around 12 improved sown species/cultivars, sometimes reaching 20 species/cultivars (Crespo, pers. com.). Many of the sown species lose dominance in the first three years after installation, and even disappear (e.g. *Trifolium incarnatum* and *Lolium multiflorum*). In intensively grazed SBPPRL installed soils with a proper managed chemical fertility, sown species rarely surpass 60% of the cover, leaving plenty of space for indigenous species. In our own study, allochthonous species diversity reached 8,6 species/0,49 m^2, although the diversity of sown species was 2,0 species/0,49 m^2. Second, artificial selection narrowed

the genetic diversity of sown species. Consequently, genotypes for fairly low soil fertility conditions are not included in SBPPRL. Anyway, in task 2 we were able to prove that sown species can also have a useful value as bioindicators of soil proprieties.

Task 2 – Testing the major characteristics of SBPPRL

Researchers Involved

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Results

The expected results of this task were:

- Correlate sample species/varieties composition with environmental factors;
- Explore the utility of species/varieties as bioindicators of soil proprieties;
- Evaluate the importance and invasibility of volunteer species in SBPPRL along environmental gradients;
- Correlate sample species composition with aerial and subterranean phytomass production, C sequestration and total N;
- Study interannual species abundance fluctuations.

The SBPPRL address a classical subject in community ecology: the effects of species diversity on ecosystem function. Empirical evidence accumulated during the past decades shows that pastures sown with species diverse mixtures are more productive. Besides higher productivity, species diverse pastures seem to be less permeable to spontaneous species of low palatability and feed value, to track environmental heterogeneity and to withstand extreme climate fluctuations. In spite of its obvious agronomic relevance, the majority of the published results on pasture diversity effects on ecosystem functions were carried out on a small scale with strictly controlled experimental conditions, involving a small number of species, in homogeneous soil conditions and during short time spans. Furthermore, these studies rarely addressed the cultivars (intraspecific diversity). Yield is the only functional characteristic of the SBPPRL ever tested.

An experiment was devised to test four hypotheses concerning SBPPRL functional characteristics:

1) Do SBPPRL track the slope system (a proxy of soil heterogeneity) microenvironments array?

2) Does the same happen with interannual climate fluctuations?

3) Do SBPPRL responses to these microenvironment spatial arrays and climate fluctuations occur at the levels of sown species and *T. subterraneum* cultivars?

4) Do SBPPRL smooth soil gradients?

To explore these issues, an observational experiment was structured in Quinta da França (Portugal) between 2007 and 2010. The agricultural year of 2008-2009 was discarded from the analysis because it was exceptionally dry. The experiment included a stabilized SBPPRL (sown in 2002) and a semi-natural pasture. They were both located in the same soil catena, displayed a similar soil fertility and were heavily grazed. Three slope positions were identified: uphill, backslope and footslope. The main difference between these two pasture types was the presence of improved pasture species in the SBPPRL. These unusual experimental conditions allowed a cautious test of the formulated hypotheses.

The results confirmed the hypothesis that diversifying the sown species/cultivar colonists' pool in seed pasture mixtures appears to allow the tracking of interannual climate variability and of soil gradients. Surprisingly, sown species diversity and *T. subterraneum* cultivar diversity were complementary in this process; they promoted, respectively, the tracking of fluctuations of interannual climate and of soil gradients (Aguiar *et al*., 2011d; Aguiar *et al*., in prep.). These results are an absolute scientific novelty both for community ecology and for pasture science. The soil heterogeneity tracking was indeed translated into higher productions at the pasture scale, and in a buffering of dry matter (DM) production along soil catenas. SBPPRL also do smooth soil gradients. This conclusion emerges from the fact that beta diversity and yield spatial variations were smaller in the SBPPRL than in the semi-natural pasture. This fact is an indirect evidence that interannual yield variations in SBPPRL are smaller than in semi-natural pastures (Aguiar *et al*., in prep.).

All published empirical evidence shows that SBPPRL are more productive than semi-natural pastures. Teixeira *et al*. (2010) and Rodrigues *et al*. (2010b) proved that higher biomass yields with a lower C/N ration increase C and N soil pools. This process probably facilitates the improved species' dominance and persistence and, at the same time, excludes low productivity species. In fact, species diversity was significantly smaller in the SBPPRL due to a decrease in the number of annual low productivity indigenous species, characteristic of the *Helianthemetea* vegetation class (Aguiar *et al*., in prep.). Our experiments comparing mineral and organic fertilization and pasture installation are congruent with these observations (Aguiar *et al*., 2011d).

The assessment of natural and sown pastures production at the Mestre farm was made in an experimental field installed in October 2001. This field has been the subject of work and information previously gathered as part of a previous experimentation and dissemination project (the Agro 71 project).

In the years 2008 and 2009 two cuts were made (in April and June) with determination of pasture production by assessing biomass availability for two types of grazing fertilized pasture: natural pasture and Sown biodiverse pasture. In 2010 there was only one cut (in June), and pasture production was assessed by the availability of biomass for grazing at both conditions.

Every year soils were sampled and analyses were made in the two types of pastures in order to assess the evolution of soil chemical properties.

The results obtained in 2010 allow a comparison of the two types of pastures, but are clearly not enough to assess biomass availability for grazing. Cutting twice a year would allow to determine biomass availability in different times of the year: in the period when biomass is high enough to withstand grazing ruminants in a high stocking density (now quite common in the extensive exploitation of pastures in the Mediterranean region) and in the period of pasture regrowth that occurs occasionally if weather conditions are favorable for vegetative growth (particularly if rainfall occurs in late April and early May). In 2010 there was only one result per type of pasture, integrating the growth of pasture biomass from autumn to spring.

For the Herdade do Mestre, results seem to indicate a higher production in the sown biodiverse pasture (in the order of 20%), but they must be taken with care due to the 2010 situation (Carneiro *et al*., 2011).

Task 3.1 – Grazing Dynamics: Animal Location

Researchers Involved

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Results

The actions and expected results of this sub-task were:

- Study the territorial preferences of three domestic mammalian herbivores (cattle, sheep and horses);
- Study the annual and interannual flock movements;
- Correlate the spatial annual and interannual flock distribution with environmental variables (topography, microclimate);
- Correlate the spatial annual and interannual flock distribution with species/varieties spatial distributions i.e. feeding preferences of different grazer species in a botanically heterogeneous pasture;
- Correlate physical soil proprieties (compaction) with spatial annual and interannual flock distribution.

Domestic herbivores actively select feed resources. This selection induces heterogeneity in vegetation and contributes to an increase in species diversity, which then influences animal feed selection. The herbivores use their memory in the feed selection process. Usually they react to feed availability and quality, revisiting the areas with a more abundant and more nutritive feed. In heavily grazed pastures the pattern of herbivore concentration and spatial distribution follows the pasture productivity gradients.

The spatial distribution and the activity (movement) of cattle and sheep were followed with GPS collars in the same experimental SBPPRL used for tasks 1 and 2. Results show that spatial distribution and activity are the same for all the individuals belonging to the same species. Although cattle and sheep had gregarious feed behavior they didn't exploit

feed resources at the same time. Regression curves relating head movements of cattle and sheep along the day were distinct among this two species. Sheep activity was distributed along the day; cattle showed multiple feed peaks along the day, but also during the night (Castro and Castro, 2009).

The gathered data didn't allow a correlation between annual and interannual flock distribution and environmental variables (topography, microclimate) or species/varieties spatial distributions. Soil bulk density was high and rather uniform and was uncorrelated with spatial annual and interannual flock distribution.

Task 3.2 – Grazing Dynamics: Animal Nutritional Status and Production

Researchers Involved

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Results

The actions and expected results of this sub-task were:

- Determine the nutritional quality of the pastures;
- Determine the nutritional status of sheep (the predominant stocking of the pastures to be studied), correlating this with their grazing points and their production level.

Concerning the nutritional quality of the pastures and the nutritional status of sheep, the experimental design involved the evaluation of milk production (yield and chemical composition), the evaluation of udder morphology and the determination of metabolic parameters for the evaluation of feed adequacy to the productive capacity of the ewes.

The sheep lambing was spread throughout the year and milk production was evaluated to compare two situations: milk yield produced under grazing (March, April and May) and milk yield produced with the flock kept mainly indoors, either during Fall (under grazing supplemented with hay) or during Winter. In 2007, when the Project started, the owners of "Quinta da França" decided to mate sheep with rams from the "Lacaune" breed in order to increase milk production. This decision led to a delay in recording milk quality parameters in order to assess the influence of the new genetic patterns of the flock. With the extension of the project for one more year, data was collected during the 2010/2011 lactation (Belo *et al.*, 2011).

Analyzes performed on pasture samples showed values of 22% of crude protein (CP), 65% digestible organic matter (OM Dig) and 9.3 Mj/kg DM of metabolizable energy (ME). For hay, average values were 8.3% CP, 55.6% OM Dig and 8.2 Mj/kg DM of ME. All ewes were supplemented in the milking parlor, twice daily, with 350 g dry matter (DM) of a concentrated feed with 25% of CPand 13 Mj/kg DM of ME (Belo *et al.,* 2011).

The average length of lactation increased from 143 days on 2007/2008 to 157 days on 2009/2010 ($p<0,001$), as well as the number of milked ewes, which increased from 211 to 410. The average ewe's total milk yield was 123 liters on 2007/2008 and 159 liters on

2009/2010 ($p<0,001$) and the average ewe's milk yield at the 100th day increased from 96 liters to 116 liters ($p<0,001$). The greatest ewe's average total milk production was from ewes lambing in September (152 liters) and March (154 liters). Concerning production per ewe at the 100th day of lactation, production averaged 122 liters on March and April while, on November and December, 87 and 92 liters were obtained, respectively ($p<0,05$). The determination of blood metabolites shows that ewes are dependent on their body reserves during the nursing phase (the first 20 days of lactation) (Belo *et al.*, 2011).

Evaluation of cheesemaking ability of milk produced throughout the lambing season of 2009/2010 was obtained by studying milk behavior during coagulation using an Optigraph® coagulometer. The different patterns of curd formation, as a function of milk characteristics, agree with differences observed both at milk production level and composition, suggesting that, at a technological level, final curd firmness is distinct. Data obtained on milk coagulation ability from the 2009/2010 and 2010/2011 lactations were evaluated according to lambing season, lactation stage and type of ewe ("original" vs. F1 "Lacaune"). Coagulation ability traits were also confronted with potential cheese yield data and casein related determinations.

On milk samples collected from the last 2 lactations (2009/2010 and 2010/2011), determinations for total casein content and casein and whey profiles were performed, allowing the evaluation of the relationship of these parameters with milk composition parameters and potential cheese yield, comparing lambing seasons and lactation stages for both "original" and F1 "Lacaune" ewes (Marques *et al*., 2011c; Marques *et al*., 2011a).

The genotyping methodology to simultaneously genotype eighteen SNPs (Single Nucleotide Polymorphisms) in S1-casein (CSN1S1), S2-casein (CSN1S2), -casein (CSN2), -casein (CSN3), -lactoglobolin (LGB) and prolactin (PRL) genes in sheep was successfully established. Polymorphisms were found in the eighteenth position studied. Frequency of major alleles ranged from 50.4 % in exon 2 of the LGB gene to 99.6 in intron 2 of the PRL gene (Marques *et al*., 2011c; Marques *et al.,* 2011a).

The genotyping methodology developed is adequate to genotype ewes for genes related to milk production, milk composition and potential cheese yield. The more promising SNPs are CSN2_3 and LGB for milk production and CSN3_5 for milk production, composition and potential cheese yield (Marques *et al.,* 2011c; Marques *et al.,* 2011a).

The mammary gland morphological characteristics were not recorded, considering that the flock's population was still evolving, causing current measurements to be less valuable for future selection decisions.

Data collected until the end of the 2010/2011 lactation will allow the production of the following additional papers:

- The flock's production performance from 2007/2008 to 2010/2011. This paper will focus on milk production and composition as well as on the evolution of the ewes' body condition score throughout the lactations considered. "Original" ewes will be compared to F1 "Lacaune" ewes relative to the Fall, Winter and Spring lambing seasons. Blood metabolic profiles will assess the feeding regime's efficacy on each season and for each milk production group established through the lactations;

- Determinations for total casein content and casein and whey profiles are being performed in milk samples collected from the last 2 lactations (2009/2010 and 2010/2011). These data will allow the evaluation of their relationship with milk composition parameters and potential cheese yield, comparing lambing seasons and lactation stages for both "original" and F1 "Lacaune" ewes;
- Data obtained on milk coagulation ability will be evaluated according to lambing season, lactation stage and type of ewe ("original" vs. F1 "Lacaune"). Coagulation ability traits will also be compared with potential cheese yield data and casein related determinations.

All information collected, as well as analytical data, may be suitable to elaborate an economic study on ewe milk production as well as the potential for cheesemaking, considering the conditions of this study on the farm "Quinta da França".

Task 4 – Modelling of SBPPRL

Researchers Involved

Ana Barradas (INRB), Tiago Domingos (IST) and Ricardo Teixeira (IST).

Results

The expected result of this task was the development of a long-term organic matter model and calibration with real data.

In detail, we aimed to study how much, on average, SBPPRL increase SOM, in relation to the baseline, which are natural grasslands. SOM accumulation through an increase of SOC is the mechanism through which carbon is sequestered in grassland soils. This is particularly important for Portugal, being one of the few countries to elect the "Grassland Management" voluntary activity, in the framework of the Land Use, Land Use Change and Forestry (LULUCF) activities, now named Agriculture, Forestry and Other Land Uses (AFOLU), under Article 3.4 of the Kyoto Protocol.

To fulfil our goal, we developed a model that determines the average trend of SOM concentration in NG, FNG and SBPPRL. Our main objective was to determine the average SOM accumulation potential in each grassland system (Teixeira *et al*., 2011a; Teixeira, 2010). In general terms, the model uses a simple mass model for SOM dynamics, calibrated using field data. The model states that the mass percent balance of SOM is the difference between input and mineralization, according to Equation 1. SOM is the SOM concentration (in g_{SOM}/g_{SOIL}) at time t, K is the SOM input, and α is the organic matter mineralization rate.

$$\frac{dSOM_t}{dt} = K_i - \alpha SOM_t$$

Equation 1

The Equation 1 can be solved by integration into Equation 2:

$$SOM_t = \frac{K_i}{\alpha}\left(1 - e^{-\alpha\Delta t}\right) + e^{-\alpha\Delta t} SOM_{t-\Delta t}$$

Equation 2

According to Equation 2, the solution has a saturating exponential form. This means that SOM accumulation is limited by an upper bound, given by $\frac{K_i}{\alpha}$. This implies that, when time tends to infinity, the concentration of SOM does not depend on the initial SOM concentration (SOM_0). In pastures, if there is no land conversion or other management activities, SOM reaches a long-term equilibrium. Improvements in management (such as fertilization or sowing of different grassland species and/or varieties) create a transient state during which SOM accumulates. The increase in production increases SOM input, but mineralization has not yet adjusted to the new situation. As years pass, mineralization also increases, and a new steady-state is reached.

The parameter α is assumed independent of the grassland system, varying with factors such as temperature and humidity. K is assumed to be a function of the grassland system, at least part of the SOM input has to reflect grassland productivity. An inspection of the data showed that farms with high initial SOM still increase their SOM concentration by a relatively high percentage, regardless of the pasture type. Both these effects are captured in Equation 3, where $K_i^{'}$ is a function of grassland system) and $aSOM_0$ represents a variable part.

$$K_i = K_i^{'} + aSOM_0$$

Equation 3

Replacing Equation 3 into Equation 2, it is obtained Equation 4

$$SOM_{i,t} = \frac{K_i^{'}}{\alpha}\left(1 - e^{-\alpha\Delta t}\right) + \frac{a}{\alpha}\left(1 - e^{-\alpha\Delta t}\right) SOM_{i,0} + e^{-\alpha\Delta t} SOM_{i,t-1}$$

Equation 4

It is also tested the hypothesis that SOM dynamics for SBPPRL is different in the first year. This approach is justified by the fact that SBPPRL plots are tilled, for sowing, in the first year, and thus, there is an increased SOM mineralization for t=1. Also, in the first year plants blossom only from the seeds which were sown, and therefore SBPPRL produce less biomass than in the following years.

The model is calibrated estimating a regression equation in which SOM_t is the dependent variable and SOM_{t-1} and SOM_0 are the independent variables. Only the constant (independent) term depends on the grassland system and, in the case of SBPPRL, if it is a first year of observation. The model assumes the formulation presented in **Equation 5**.

$$\begin{aligned} SOM_t = {} & C_{1,k_{SBPPRL}^{t=1}} d_{1,k_{SBPPRL}^{t=1}} + C_{1,k_{SBPPRL}^{year>1}} d_{1,k_{SBPPRL}^{year>1}} + \\ & C_{1,k_{FNG}} d_{1,k_{FNG}} + C_{1,k_{NG}} d_{1,k_{NG}} + \\ & C_2 SOM_0 + C_3 SOM_{t-1} \end{aligned}$$

Equation 5

The parameters of the equation can, therefore, be calculated using the system of equation presented below.

$$\begin{cases} \alpha = \dfrac{-\ln C_3}{\Delta t} \\ a = \dfrac{\alpha C_2}{1 - e^{-\alpha \Delta t}} \\ K_i^{'} = \dfrac{\alpha C_{1,i}}{1 - e^{-\alpha \Delta t}}, i = \{SBPPRL(t=1), SBPPL(t>1), FNG, NG\} \end{cases}$$

The regression constants are estimated using software SPSS Statistics 17.0 with an Ordinary Least Squares method and a stepwise regression, described below. The data used to calibrate the model was obtained from rainfed pastures in eight farms in Portugal.

Results for the stepwise regression showed that the statistical fit to base data of the model, measured with the adjusted-R^2 is high, and corresponds to a low root mean standard error (rMSE). The results also indicate that both the first-year dummy for SBPPRL, and the dummy for NG were not significant. We started by removing the least significant one, the first-year dummy for SBPPRL and then the dummy for NG. The new results also show no significance for the NG dummy. This means that fertilized plots do not increase their SOM pool significantly more than non-fertilized plots. Therefore, we obtained a final model with only three regression constants significantly different from zero: one regarding SBPPRL specific SOM input, one referring to the parameter SOM_0 (C_2), and one referring to the mineralization rate (C_3).

It is possible to convert the increase of SOM in the increase of CO_2. SOM is composed of living organisms (bacteria, fungi, plant roots and animals), dead animals and plant tissues in several stages of decomposition but still recognizable, and a complex mixture of decomposed, modified or reprocessed material called humus. So, basically, SOM is composed of C, N and O. Actually, 58% of SOM is carbon (IPCC, 1997, 2003). It is possible to use this conversion, along with the soil bulk density in Portuguese soils, 1,48 $g.cm^{-3}$ according to the Harmonized World Soil Database (HWSD; Fisher *et al.,* 2008); the depth at which the sample was collected; the molecular weight of carbon dioxide and the atomic weight of carbon to convert SOM to t $CO_2.ha^{-1}$.

We find that the expected steady-state long term SOM concentration in undisturbed SBPPRL is higher than in NG and FNG. In 10 years, there is an average increase of 0.19-0.20 percentage points per year in SBPPRL (equivalent to 1.33-1.40 t $C.ha^{-1}.yr^{-1}$ or 1.48-1.56 t $C.ha^{-1}.yr^{-1}$, depending on the soil density). In turn, SOM increases in FNG and NG are respectively 0.13-0.14 (0.94-0.95 t $C.ha^{-1}.yr^{-1}$ or 1.05-1.07 t $C.ha^{-1}.yr^{-1}$) and 0.07-0.08 percentage points per year (0.51-0.53 t $C.ha^{-1}.yr^{-1}$ or 0.57-0.59 t $C.ha^{-1}.yr^{-1}$). SBPPRL induces SOM increase due to sowing and fertilization, but mineralization rates are equal to those from FNG. NG has stable SOM pools, with lower input and mineralization rates.

Task 5 – Soil monitoring in a large set of natural and sown pastures

Researchers Involved

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Results

The expected results of this task were:

- Evaluate the evolution of annual carbon sequestration by both sown and natural pastures (fertilized) in different soil types and at different soil depths;
- Continue the building of a long-term series of soil organic matter at eight points, each with natural and sown pasture;
- Evaluation of the evolution of annual carbon sequestration by both sown and natural pastures (fertilized) in different soil types and at different soil depths;
- Characterization of stocking rates at sown and natural pastures;
- Use monitoring data gathered by farmers for a wider sampling (albeit with less precision).

Collection of samples was foressen in the following farms: Quinta da França, Herdade das Refroias, Herdade do Mestre, Herdade da Cabeça Gorda, Herdade da Torre e do Cinzeiro e Herdade dos Claros Montes. Samples were to be collected once a year in eight sown and eight natural pastures between 2007 and 2009. Besides these we also gathered additional data in 6 Portuguese farms and 116 additional sample points.

The types of samples to collect were:

- Composite soil samples in the three years of the project from 0 to 10 cm, 10 to 20 cm and 20 to 30 cm;
- Undisturbed soil samples in the first year of the project;
- Samples of plant material.

Plot areas ranged from 5 to 15 ha. Each plot's soil and landscape type was approximately homogeneous, in terms of soil and previous use. These pastures were no isolated test sites. They are located in private land currently used by farmers for animal production. Considering the fertilization, both grassland systems were subjected to the same fertilization rates during all years. The difference during the installation of SBPPRL is that, previous to sowing, plots are tilled in the upper layer of soil, and a phosphate and potassium fertilizer is used (Teixeira *et al.*, 2011a; Teixeira, 2010).

In 2007 samples were collected in the six farms, but it was found that in Herdade da Torre e do Cinzeiro the pastures were gone, so it was decided not to monitor this farm in subsequent years. Therefore, in 2008 five farms were monitored. In 2009 it was only possible to monitor one farm (Quinta da França) due mainly to weather conditions

observed in the period for sample collection. In total, six natural pastures, six sown pastures and two natural pastures fertilized were monitored.

It was decided that the depth of sampling should be changed to composite samples to 0-10 cm and 0-30 cm, to allow direct comparison of the data with the data obtained in the Agro 71 Project in the same pastures; this decision also implied that for the purposes of evaluation of soil carbon stocks the information collected in these samples would be sufficient.

The collection of undisturbed samples was postponed for the second year of the project, because it implied the support of landowners, which would be difficult to obtain in the first year of the project. In terms of the quality of results, this delay did not cause any problem, because the density of a soil is a feature where changes occur only in the long term.

The samples of plant material were never collected during the project, because during the soil sample collection period the pastures were not able to be sampled.

All composite soil samples were analyzed and the following parameters were determined: macronutrients, micronutrients, base exchange, organic carbon, organic matter and pH. Bulk density in the undisturbed samples was also determined.

The results from the monitoring of soil organic matter (and therefore, carbon sequestration) show that the minimum SOM increase for SBPPRL was obtained in Coruche, where the pasture blooming after the first establishment was poor. The highest increases for SBPPRL were obtained in the more productive gneiss soil in Vaiamonte and the schist soils of Herdade de Refróias. In Herdade de Refróias, SOM at the beginning was already 3%. The results show a higher increase in SOM (and therefore, carbon sequestration) for SBPPRL (Teixeira *et al*., 2011a; Teixeira, 2010). The increased input in SBPPRL is due to two factors. First, production responds to fertilization, even though this effect alone is not enough to significantly increase the SOM pool, since results for FNG and NG are similar. Second, production responds to the improved seed bank independently of soil characteristics (Teixeira *et al*., 2011a; Teixeira, 2010; Teixeira and Domingos, 2010; Teixeira *et al*., 2008c; Teixeira *et al*., 2008d; Teixeira *et al*., 2008b).

We also established a pasture management recommendation guidelines, as presented in IST (2011). These guidelines are annexed to this scientific report.

Task 6 – Integrated assessment of SBPPRL

Researchers Involved

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Results

The expected results of this task were:

- Improve the Life Cycle Assessment (LCA) method regarding its application to agriculture, namely solving some of its shortcomings related to soil and water;
- Adapt the LCA method to the Portuguese context, analysing aggregation factors and emission and damage databases;
- Develop methods to quantify economically the impacts obtained in 1;
- Combine these methods in the analyses of SBPP;
- Assess internal costs and analyse the trade-offs between these and external costs;
- Account for the complete balance of Greenhouse Gases associated with SBPP systems: carbon sequestration by the pasture; lower indirect emissions induced by agriculture and animal production, due to a reduction on the needs of animal feed and nitrogen fertilizer; reduced forest fire risk; increased animal CH_4 and N_2O emissions, due to the higher stocking rate attained; increased soil N_2O emissions by legumes, increased CO_2 emissions due to liming.

Since the beginning of the domestication of livestock by humans, fields of herbaceous plants, named in this sense as "pastures" or "grasslands", have been used to feed animals. In Portugal, there are now around 1.5 million hectares of grasslands (INE, 2010), divided between three grassland systems: natural grasslands (NGs), fertilized natural grasslands (FNGs), and SBPPRLs. Given their characteristics, these last ones are believed to have a better environmental, economic and social performance, when compared to natural grasslands (both NG and FNG). In this task we aimed to develop an integrated assessment of the SBPPRL.

As showed in Figure 1, SBPPRL allow a higher increase in soil organic matter (SOM) when compared to natural grasslands. This represents a particularly important impact since Mediterranean soils and semi-arid countries show low SOM concentration. An increase on SOM is directly related to an increase in soil organic carbon, which represents, for the SBPPRL, a sequestration of about 5 t $CO_2.ha^{-1}.yr^{-1}$ (Teixeira *et al*., 2011a; Teixeira, 2010; Teixeira and Domingos, 2009b; Teixeira *et al*., 2008d; Teixeira *et al.*, 2008c) However, in order to have an overall balance, it is necessary to subtract the emissions from animals, legumes and liming. The balance is still positive – 4.1 t $CO_2.ha^{-1}.yr^{-1}$ sequestered (Teixeira *et al*., 2008b; Teixeira *et al*., 2008a; Teixeira *et al*., 2008c). Higher levels of SOM also imply an increase on water retention, which is important for flood regulation, decreasing the processes of soil erosion.

Increasing SOM also improves soil nutrient availability and therefore plant productivity. Related to the increase on productivity there is also an increase in the stocking rates: on average the stocking rate of SBPPRL is twice that of natural pastures (Teixeira, 2010). The higher stocking rate represents an advantage in terms of economic viability, since, for the same area it is possible to raise more animals, but also because the need for concentrated feeds decreases. Since the pastures are actively grazed, there is no invasion

by shrubs. It should also be noticed that, since SBPPRL allow a decrease on commercial feed required and therefore, a decreased maize area (maize is a major component of commercial feed), this area can be used to other proposes. According to Gonçalves *et al.* (2011), and in a Life Cycle Approach, it is environmentally favourable to use that area to produce biomass that will replace coal in the production of electricity.

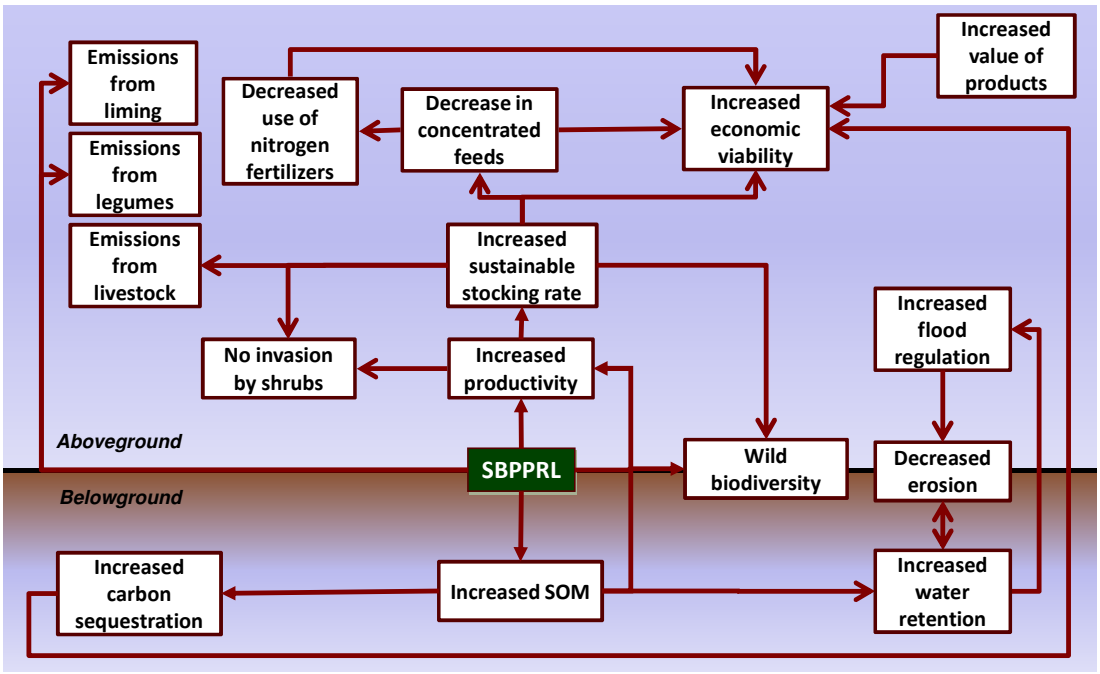

Figure 1 – An integrated approach to SBPPRL (Teixeira, 2010)

In order to quantify the environmental indirect and direct impacts we used a Life Cycle Approach, and compared the SBPPRL with the natural grasslands. Since SBPPRL are more intensive in terms of inputs (namely fertilizers), an analysis per hectare shows that the SBPPRL are only favourable regarding the greenhouse gases emission. However, as SBPPRL are much more productive and allow a higher stocking rate, when the assessment takes this into account, the SBPPRL are favourable for every impact category analysed (Teixeira, 2010; Rodrigues, 2008).

Up to this point, we proved that SBPPRL are better than the natural pastures. However, they can be worse than other land uses. Given its importance, we analysed the case of maize production, both to concentrated feed and bioethanol production. Our results showed that there is also an environmental advantage in sowing irrigated pastures, when compared to maize. For all impact themes, the impact of a scenario where farmers sown part of their farm with SBPPRL to replace commercial feeds is lower. The only significant impact of SBPPRL is the production of phosphate fertilizer. When the previous analysis is included in a fuel perspective, with the production of bioethanol from maize as opposing to the installation of pastures and use of gasoline, the results are also favorable to the pastures (Teixeira, 2010; Valada *et al.,* 2008c; Valada *et al.*, 2008a).

Regarding the social and economic analysis, we concluded that the changing structural conditions that take place with this project, namely regarding the economic (increased revenue) and social (information and knowledge) aspects of rural zones, will positively influence the adoption of sustainable land uses (Teixeira, 2010; Teixeira, 2008; Teixeira *et al.,* 2007a).

If SBPPRL are indeed a good option for farmers, with many economic and environmental advantages, then it is somewhat puzzling at first sight that the rate of their implementation has been decreasing. There are many possible explanations for the fact that some farmers stop installing pastures and other farmers never install them at all. Many of them have to do with social reasons, namely cultural barriers. There seems to be an intuitive idea amongst farmers that annual pastures are more productive than permanent pastures, even though annual pastures are more expensive and do not show better results. This idea is persuasive because farmers are economic agents sensitive to social representations of their activity, and believe that showing soil management implies actively performing actions such as sowing every year (João Paulo Crespo, personal communication). One dramatic example of this are most parts of the Trás-os-Montes region, where farmers are socially well-regarded as good soil managers if they keep grasslands completely safe from infestants all year round. They achieve this by recurring to very frequent tillage, sometimes as often as two or three times a year (António Martelo, personal communication). Due to the slope of most plots in the region, this accelerates soil loss and, in the medium run, greatly decreases productivity. Keeping a permanent pasture also implies good management, but in a less visible way, and thus the social perception of the activity of the farmer is harder (Teixeira, 2010; Teixeira, 2008).

We must also note that SBPPRL is a relatively new and innovative system, which implies some knowledge transfer not only to break social barriers, but also to guarantee that farmers manage the pastures correctly. Financially, the agricultural sector faces risks that the farmer does not control (price fluctuation of inputs and of products sold, climate, among others), even for activities that the farmer dominates. Uncertainty is even higher for innovations, and so farmers are more risk adverse (Teixeira, 2010).

Assessment

Table 1 presents the performance of the project, as compared to the indicators foreseen. The goal in terms of published scientific papers is very ambitious and the project has not yet been totally successful in this concern. However, six papers are still waiting for response to submission requests that, if positive, will end in the publication of 14 international peer-reviewed papers (which was the main goal of the project in terms of publications). Additionally, the project had a much larger than expected attainment of the goals for PhD and MSc dissertations and presented a large number of communications and posters. It also received several awards (see “Aims Reached”).

Table 1 – Performance indicators (from 2007/08/01 to 2011/01/31).

Descrição	Foreseen	Carried Out Published or accepted for publication (submitted)
A – Publications		
Books	0	1 (0)
Book chapters	0	1 (0)
Papers in international journals	14	8 (6)
Papers in national journals	6	7 (1)
B – Presentations		
In international scientific meetings	17	10
In national scientific meetings	9	10
C – Reports		
D – Organization of seminars and conferences		
E – Advanced Training		
Ph.D. theses	1	2
M.Sc. theses	2	4
Other		
F – Models		
G - Software		
H – Pilot Installations		
I – Prototypes		
J – Patents		
L – Other (Posters)		11

Table 2 – Papers in international journals

Paper	Journal	URL
Aguiar, C., Fernández-Núñez, E., Pires, J. M., Rodrigues, M.A., Domingos, T. (2011a). The effects of climate fluctuations and soil heterogeneity on the floristic composition of natural and sown Mediterranean annual pastures.	Submitted.	http://sae.ist.utl.pt/~tdomingos/AguiarDomingos2011.pdf
Aguiar, C., J. Pires, M. A. Rodrigues, M. E. Fernández-Nuñez (2011b). The effects of climate fluctuations and soil heterogeneity on the floristic composition of sown Mediterranean annual pastures.	Journal of Water and Land Development (accepted for publication).	http://sae.ist.utl.pt/~tdomingos/AguiarPires2011a.pdf
Aguiar, C., Pires, J. M., Rodrigues, M. A., Fernández-Núñez, E. (2011d). Effects of sowing and fertilisation in the establishment of annual legume rich permanent pastures.	Grassland Science in Europe (accepted for publication).	http://sae.ist.utl.pt/~tdomingos/AguiarPires2011b.pdf
Belo, A. T., M. R. Marques, S. Bernardino, J. M. Ribeiro, C. C. Belo (2011). Sheep milk: yield, composition and potential cheese yield.	Journal of the International Dairy Federation (accepted for publication).	http://sae.ist.utl.pt/~tdomingos/BeloMarques2011.pdf
Gonçalves, M., T. Valada, T. Domingos (2011). *Eucalyptus globulus* as raw material for electricity generation - A life cycle assessment including indirect land use change.	Submitted.	http://sae.ist.utl.pt/~tdomingos/GoncalvesDomingos2011.pdf
Marques, M. R., A. T. Belo, J. M. Ribeiro, S. Bernardino, T. Domingos, C. C. Belo (2011a). Influence of casein genotypes on potential cheese yield in dairy ewes.	Submitted.	http://sae.ist.utl.pt/~tdomingos/MarquesDomingos2011.pdf
Marques, M. R., F. Sobral-Matos, A. Nunes, S. Bernardino, J. M. Ribeiro, C. C. Belo (2011c). Simultaneous genotyping of eighteen single nucleotides polymorphisms at casein, beta-lactoglobulin and prolactin genes in sheep.	Submitted.	http://sae.ist.utl.pt/~tdomingos/MarquesMatos2011.pdf
Rodrigues, M. A., V. Gomes, L. G. Dias, J. Pires, C. Aguiar, M. Arrobas (2010b). Evaluation of soil nitrogen availability by growing tufts of nitrophilic species in an intensively grazed biodiverse legume-rich pasture.	Spanish Journal of Agricultural Research 8(4), 1058-1067.	http://sae.ist.utl.pt/~tdomingos/RodriguesGomes2010.pdf
Teixeira, R., Domingos, T., Canaveira, P., Avelar, T., Basch, G., Belo, C.C., Calouro, F., Crespo, D., Ferreira, V.G., Martins, C. (2008a). Carbon sequestration in biodiverse sown grasslands.	Options méditerranéenes – Sustainable Mediterranean Grasslands and Their Multi-Functions, A-79: 123-126.	http://sae.ist.utl.pt/~tdomingos/TeixeiraDomingos2008a.pdf
Teixeira, R., Domingos, T., Costa, A.P.S.V., Oliveira, R., Farropas, L., Calouro, F., Barradas, A.M., Carneiro, J.P.B.G. (2008c). The dynamics of soil organic matter accumulation in Portuguese grasslands soils.	Options méditerranéenes – Sustainable Mediterranean Grasslands and Their Multi-Functions, A-79: 41-44.	http://sae.ist.utl.pt/~tdomingos/TeixeiraDomingos2008b.pdf

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Valada, T., R. Teixeira, T. Domingos (2011). Production of maize to bioethanol in Portugal - a Life Cycle Approach including indirect land use change.	Submitted.	http://sae.ist.utl.pt/~tdomingos/ValadaDomingos2011.pdf

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