

# **Fijación biológica de nitrógeno**

**Juan A. Fernández**

- Introducción
- Proyecto Eurolegume
  - Fijación biológica del N
  - Disponibilidad N en cultivos posteriores
  - Emisión de gases invernadero ( $N_2O$ )

# **¿Qué proporcionan a los humanos?**

**Alimentos ricos en proteínas**

**Reducen los aportes de N en agricultura**

**Mejoran propiedades del suelo**

**Reducen plagas y enfermedades**

**Papel en la sustentabilidad ambiental**  
**Reducen las emisiones de los gases de invernadero**  
**Contribuyen al secuestro de C en los suelos**  
**Reducen la energía fósil usada en la producción de alimentos**

**Reducción de los aportes de N**  
**Contribuyen a la fertilidad del suelo**  
**Captan N atmosférico a través de una simbiosis**  
**con bacterias y dejan N para los cultivos**  
**Reducen la contaminación de agua**

# **Reducen las emisiones de los gases de invernadero ( $\text{CO}_2$ , $\text{N}_2\text{O}$ y $\text{CH}_4$ )**

## **Directamente**

## **Indirectamente (producción y transporte)**



**Contribuyen al secuestro de C  
Residuos de las plantas son emitidos como CO<sub>2</sub> por los microbios  
Acumulan C orgánico en formas estables  
Reducen emisiones de CO<sub>2</sub>**





**EUROLEGUME**  
2014-2017

# Enhancing of legumes growing in Europe through sustainable cropping for protein supply for food and feed

FP7 Research Project № 61378



Funded by the 7th Research Framework  
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[www.eurolegume.com](http://www.eurolegume.com)

# Enhancing of legumes growing in Europe through sustainable cropping for protein supply for food and feed

FP7 Research Project Nº 61378

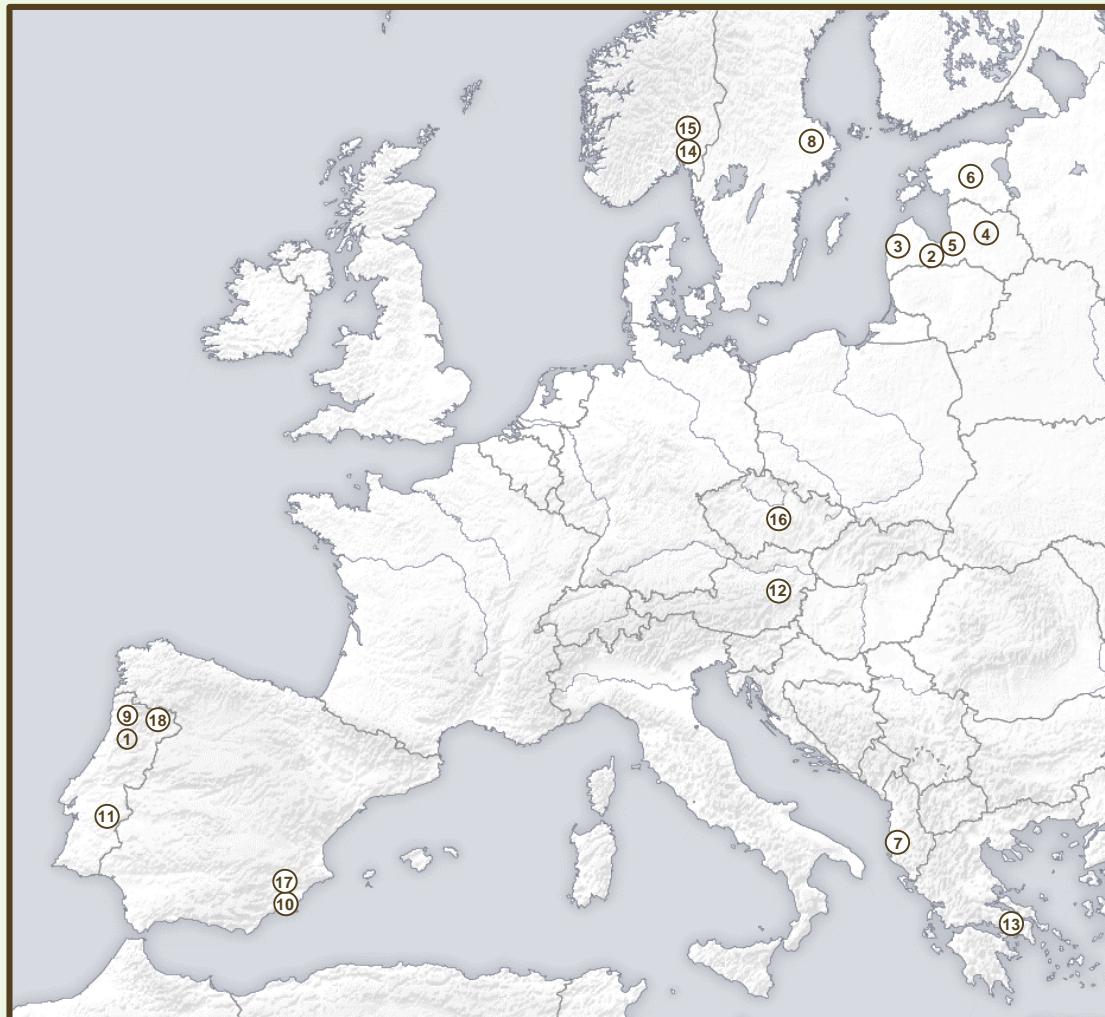
- **12 Research Institutions**
- **6 SME (5 SME and 1 SME performing RTD)**
- **UTAD for Project management and coordination**
- **ECRI for Communication an Dissemination**

## Budget Breakdown

- **Total investment of 6,5M€**
- **Requested EU contribution of 5M€**
- **4-year project starting January, 1<sup>st</sup>, 2014.**

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## Location of the 18 EUROLGUME partners

- |                       |            |
|-----------------------|------------|
| ① UTAD                | ⑩ UPCT     |
| ② LLU                 | ⑪ INIAV    |
| ③ PHRC                | ⑫ BOKU     |
| ④ SPPBY               | ⑬ AUA      |
| ⑤ BIOEFEKTS           | ⑭ BIOFORSK |
| ⑥ ECRI                | ⑮ UMB      |
| ⑦ AUT                 | ⑯ SYMBION  |
| ⑧ JTI                 | ⑰ KPRA     |
| ⑨ FRESCURA<br>SUBLIME | ⑱ ESTIRPE  |



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## WP 1 – Project Management & Coordination

**T1.1 Planning and Scheduling**

**T1.2 Progress monitoring, control & quality management**

**T1.3 Implementing the structure for communication, management & administration**

**T1.4 Funding distribution for EC payments**

**T1.5 Project quality plan**



WP Leader: Prof. Dr. Eduardo Rosa | UTAD

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## WP 2 – Broading of genetic diversity in breeding through evaluation of genetic resources

Description of Biochemical/morphological features of pea, faba bean, and cowpea/black-eyed bean genotypes to develop varieties for food/feed and use in breeding

**T2.1 Sourcing and characterization of genetic diversity**

**T2.2 Phenotyping selected accessions for site specific biotic and abiotic stress**

**T2.3 Creating of Near Infrared Reflectance (NIR) spectroscopy calibration for selected quality parameters of peas**

**T2.4 Evaluation of genetic diversity by molecular methods**



# Evaluation of genetic resources



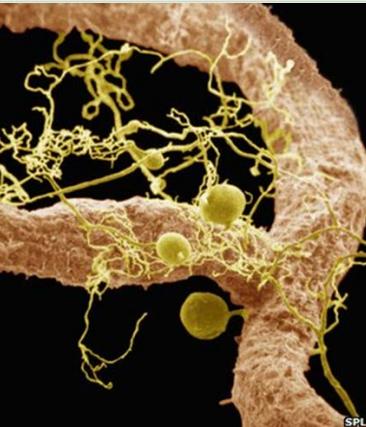
# Evaluation of genetic resources





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## WP 3 – Selection of appropriate rhizobium strains to support nitrogen fixation and development of inoculants

Selection of the most appropriate *Rhizobium* and arbuscular *mycorrhizae fungi* to support nitrogen fixation. Development of new commercial inoculants

**T3.1 Selection of rhizobial strains and arbuscular mycorrhizal fungi (AMF) for enhanced BNF and legume growth**

**T3.2 Phenotypic and genotypic characterization of *Rhizobium leguminosarum* and *Bradyrhizobium* spp. Strains**

**T3.3 Development of new commercial products**  
Formulation for pea and fava bean  
Formulation for cowpea

WP Leader: Dr. Guilhermina Marques | UTAD



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## WP 4 – Nutritional value and innovative food and feed

Develop foods and feeds based on the nutritive value from European varieties of pea, faba bean, and cowpea



**T4.1 Evaluation of the quality of new feed and food**

**T4.2 Multiuse of all three species in development of innovative foods/feeds**

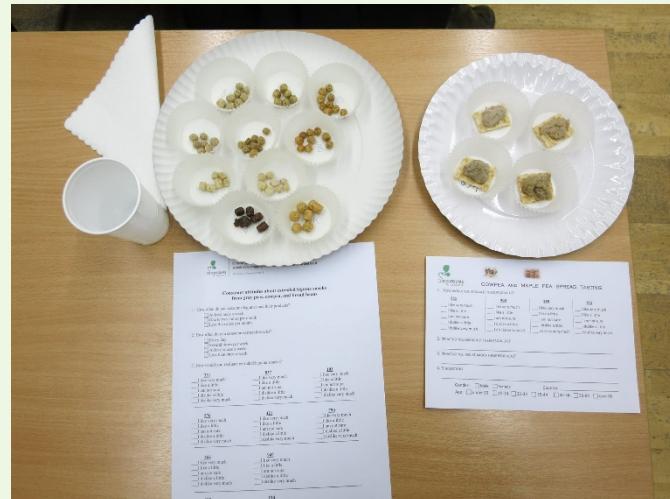
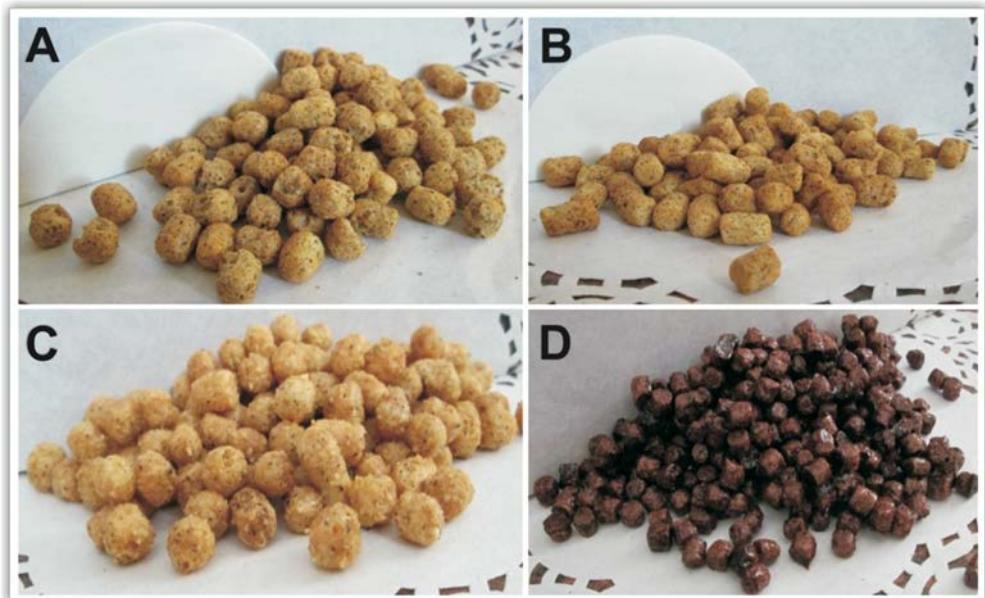
**T4.3 Development of new processing and packing techniques**

**T4.4 Economic assessment of new products**

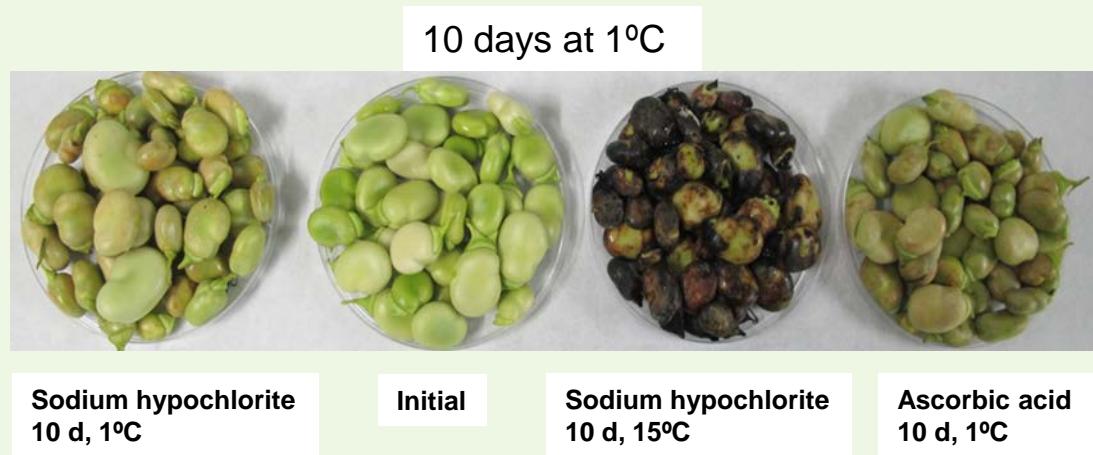
**WP Leader: Dr. Ruta Galoburda |**

**LLU**

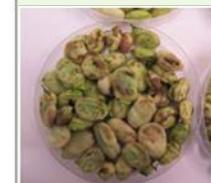
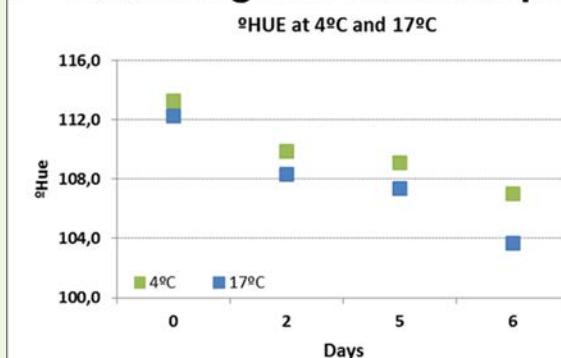
# Nutritional value and innovate food and feed



# Nutritional value and innovate food and feed



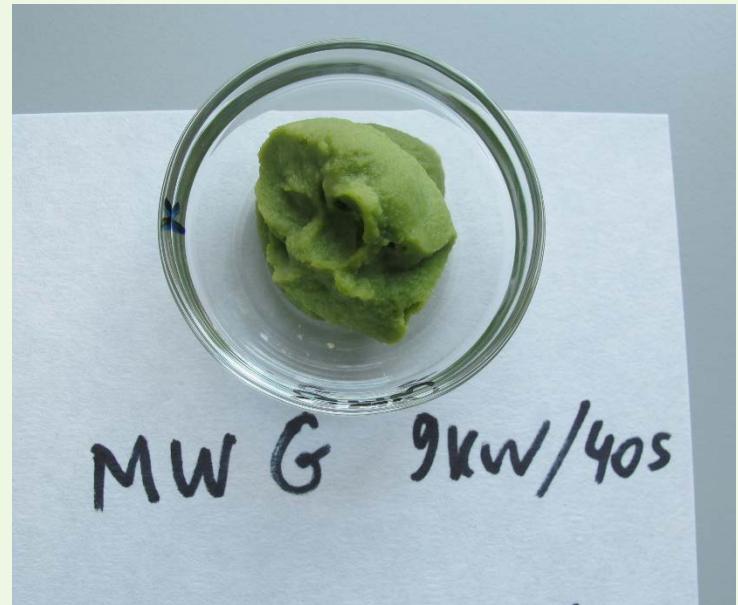
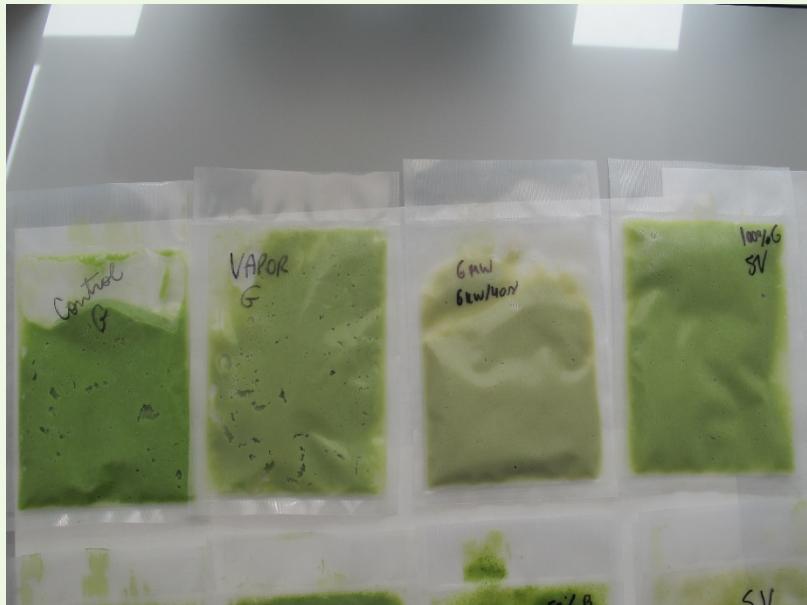
Browning was the main problem



# Nutritional value and innovate food and feed



# Nutritional value and innovate food and feed



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## WP 5 – Legume supported cropping system in sustainable agriculture



Introducing of pea, faba bean, and cowpea in production schemes to enhance the sustainability of agricultural systems and improve the yield and economical benefit

### T5.1 High protein outcome-focused field investigations

### T5.2 Biological nitrogen fixation focused investigations

### T5.3 Transcriptomic characterization in drought stress

**WP Leader: Prof. Dr. Dimitrios Savvas | AUA**



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## WP 5 – Legume supported cropping system in sustainable agriculture



### T5.2 Biological nitrogen fixation focused investigations

- Sub-Task 5.2.1. Detection of genotypes & cropping system influence on soil properties
- Sub-Task 5.2.2. Evaluation of the BNF efficiency of different Rhizobia & AMF strains under field conditions
- Sub-Task 5.2.3. Evaluation of commercially produced Rhizobia & AMF strains in pot trials and under field conditions

**WP Leader: Prof. Dr. Dimitrios Savvas | AUA**



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## WP 5 – Legume supported cropping system in sustainable agriculture

### T5.2 Biological nitrogen fixation focused investigations



#### **$^{15}\text{N}$ natural abundance method**

$$\delta^{15}\text{N} = \frac{\text{Sample atom}\%^{15}\text{N} - 0.3663}{0.3663} \times 1000$$



$$\%Ndfa = \frac{\delta^{15}\text{N of reference plant} - \delta^{15}\text{N of } N_2\text{-fixing legume}}{\delta^{15}\text{N of reference plant} - B} \times 100$$

$$BNF_{hb} = \frac{DMY \times NL \times \%Ndfa}{10000}$$

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## WP 5 – Legume supported cropping system in sustainable agriculture



### Sub-Task 5.2.2. Evaluation of the BNF efficiency of different Rhizobia & AMF strains under field conditions

- 2014-2015. Haba: *Burkholderia* sp. + *Glomus intraradices*, *G. etunicatum*, *G. claroideum* y *G.mosseae* (AMF)
- 2015. Caupi: R32 *Rhizobium* sp., R63 *Bradyrhizobium japonicum*, R57 *Bradyrhizobium* sp. + AMF
- 2015-2016. Haba: F11.1 *Rhizobium leguminosarum* bv. *viciae*, F33.3- *Burkholderia cenocepacia*, R46- *B. vietnamiensis* + AMF

**WP Leader: Prof. Dr. Dimitrios Savvas | AUA**



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## WP 5 – Legume supported cropping system in sustainable agriculture



**WP Leader: Prof. Dr. Dimitrios Savvas | AUA**



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## Rendimiento de un cultivo de haba inoculado con bacterias fijadoras de nitrógeno y micorrizas

Rendimiento (kg/m <sup>2</sup> )			
Muchamiel		Palenca	
control	inoculado	control	inoculado
1,2	1,9 (58%)	1,3	2,3 (77%)

Y	X	R <sup>2</sup>	F value
% de micorrización	CE ( $\mu\text{S cm}^{-1}$ )	0.99	45***
	B-glucosaminidasa ( $\mu\text{mol PNP g}^{-1} \text{h}^{-1}$ )		
	Producción (kg ha <sup>-1</sup> )	0.91	253***
Peso de 100 semillas	Nt suelo (g kg <sup>-1</sup> )	0.99	308***
	BNF nódulos (kg N ha <sup>-1</sup> )		
Número de nódulos	Deshidrogenasa (nmol INTF g <sup>-1</sup> h <sup>-1</sup> )	0.81	19***
	BNF nódulos (kg N ha <sup>-1</sup> )		
BNF raíz (kg N ha <sup>-1</sup> )	P raíz (mg kg <sup>-1</sup> )	0.76	15***
	NO <sub>3</sub> <sup>-</sup> suelo (mg kg <sup>-1</sup> )		

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## WP 5 – Legume supported cropping system in sustainable agriculture

### Sub-Task 5.2.3. Evaluation of commercially produced Rhizobia & AMF strains in pot trials and under field conditions



#### Summer 2016

- 2 pea accessions
- different in root architecture and morphology
- 2 nitrogen fixing-bacteria + AMF
- Pots fill with field soil mixed with 30% sand

WP Leader: Prof. Dr. Dimitrios  
Savvas | AUA



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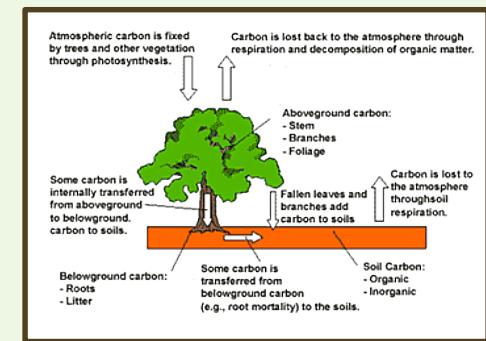
## WP 6 – Management and valorisation of the residual biomass

To give an added-value to products from legume grain production

### T6.1 Added value of crop residues

### T6.2 N – availability to following crops

### T6.3 Carbon sequestration and greenhouse gases balance



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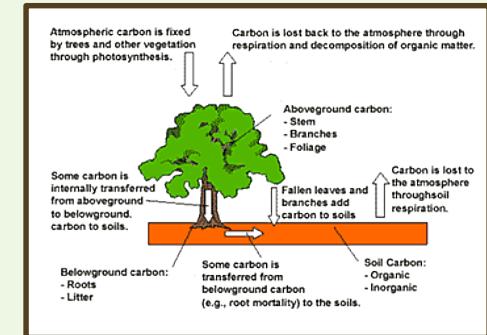
## WP 6 – Management and valorisation of the residual biomass

### T6.2 N – availability to following crops

**Table 6.** Soil analysis after a melon crop in rotation with faba bean crop.

Previous cultivar (PC)	Management practice (MP)	Total N in soil (g/kg)	Soil organic carbon (g/kg)	NH <sub>4</sub> <sup>+</sup> in soil (mg/kg)
Muchamiel	Conventional	1.00±0.05	13.1±0.4	6.1±1.7
Palenca	Conventional	0.90±0.29	18.1±6.7	4.5±0.5
Fallow plot	Conventional	1.00±0.13	13.4±1.7	7.6±1.1
Muchamiel	Organic	0.98±0.11	11.9±1.1	7.7±2.0
Palenca	Organic	0.84±0.16	11.8±1.1	5.8±0.3
Fallow plot	Organic	0.98±0.06	12.5±0.5	5.7±0.6
<i>Significance</i>				
PC		1.030 <sup>N.S.</sup>	0.318 <sup>N.S.</sup>	0.675 <sup>N.S.</sup>
MP		0.187 <sup>N.S.</sup>	3.926 <sup>N.S.</sup>	0.004 <sup>N.S.</sup>
PC x MP		0.024 <sup>N.S.</sup>	0.051 <sup>N.S.</sup>	2.711 <sup>N.S.</sup>

Table 7. Crop yield and fruit quality parameters in the melon crop in rotation with faba bean crop.					
Previous cultivar (PC)	Management practice (MP)	Crop yield (kg/ha)	Fruit weight (kg)	Fruit length (cm)	Sugar content (%)
'Muchamiel'	Conventional	20060±9786	2.50±0.14	25.2±1.6	9.05±0.07
'Palenca'	Conventional	13000±6307	2.14±1.43	22.3±4.6	10.05±1.76
Fallow plot	Conventional	16667±7761	2.35±0.44	24.0±1.5	9.06±0.68
'Muchamiel'	Organic	15710±7877	2.10±0.70	21.3±3.8	9.45±0.21
'Palenca'	Organic	19540±735	2.10±1.10	18.2±5.8	8.30±0.90
Fallow plot	Organic	14486±5302	2.13±0.64	23.3±1.73	9.20±1.71
<i>Significance</i>					
PC		0.143 <sup>N.S.</sup>	0.660 <sup>N.S.</sup>	1.491 <sup>N.S.</sup>	0.012 <sup>N.S.</sup>
MP		0.002 <sup>N.S.</sup>	1.242 <sup>N.S.</sup>	2.682 <sup>N.S.</sup>	0.407 <sup>N.S.</sup>
PC x MP		0.657 <sup>N.S.</sup>	0.173 <sup>N.S.</sup>	0.453 <sup>N.S.</sup>	1.052 <sup>N.S.</sup>

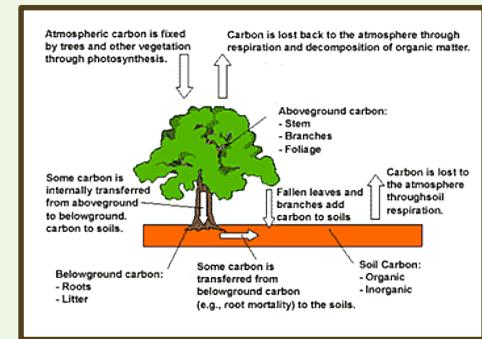
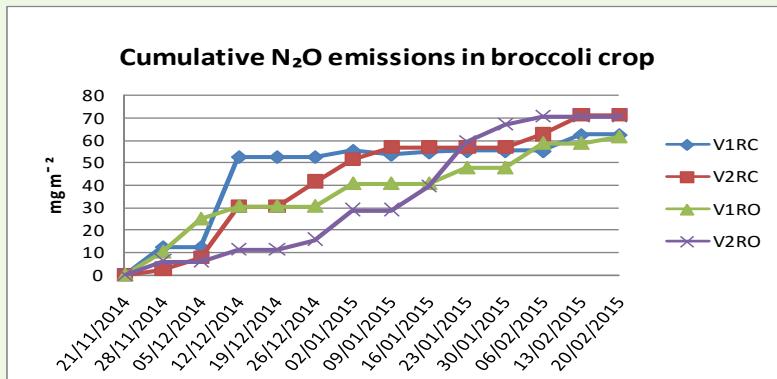
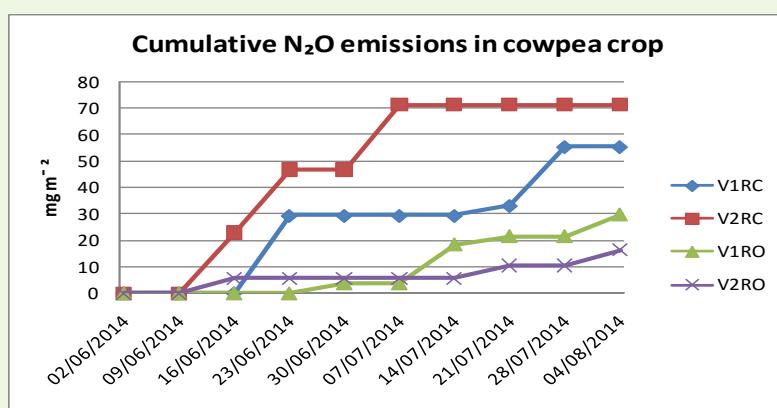


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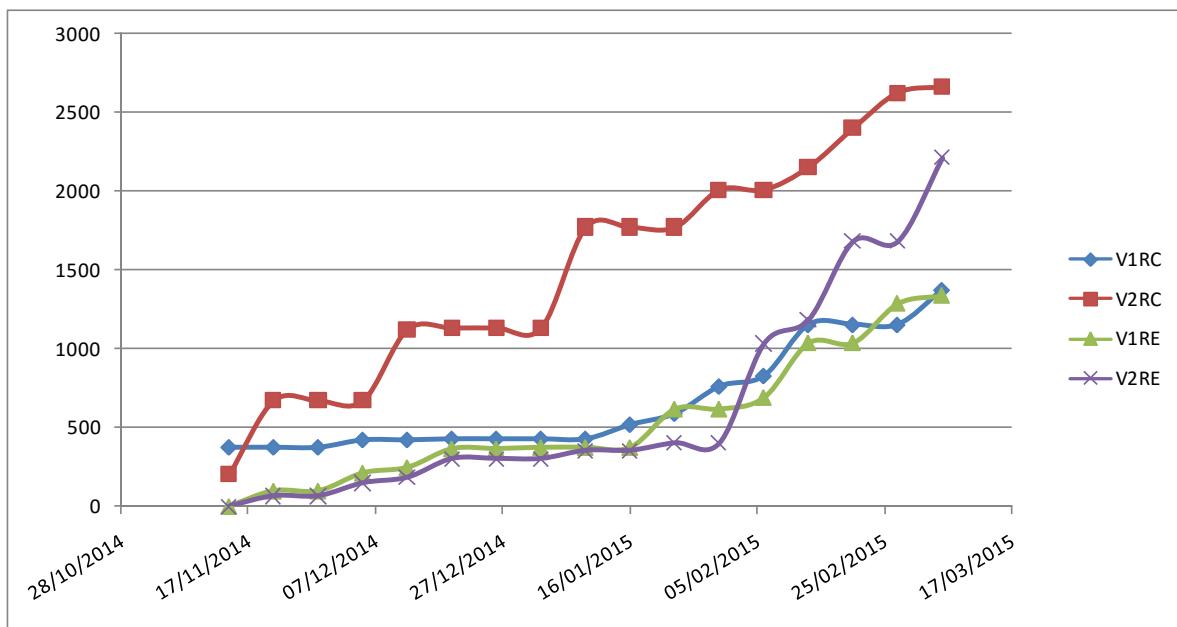
## WP 6 – Management and valorisation of the residual biomass

### T6.3 Carbon sequestration and greenhouse gases balance



## Emisión de gases de efecto invernadero – CH<sub>4</sub>

CH <sub>4</sub> (mg/m <sup>2</sup> )	
Muchamiel (V1)	Palenca (V2)
Un incremento de <b>33,6 mg/m<sup>2</sup></b> en cultivo convencional (RC) frente al ecológico (RE)	Un incremento de <b>455 mg/m<sup>2</sup></b> en cultivo convencional (RC) frente al ecológico (RE)





**EUROLEGUME**  
2014-2017

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## WP 7 – Communication and Dissemination

### T7.1 Development of website and its updating

### T7.2 Planning and dissemination activities

### T7.3 Implementation of dissemination activities

- i) Publication of scientific papers
- ii) Dissemination of activities throughout newsletters and social networks
- iii) Publication of EUROLGUME results and outcomes

[www.eurolegume.com](http://www.eurolegume.com)

WP Leader: Dr. Margit Olle | ECRI

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## Thank you very much



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