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**ANNUAL SELF-RESEEDING LEGUMES
AND THEIR APPLICATION INTO
MEDITERRANEAN CROPPING SYSTEMS**

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إلى أمي و أبي

لكل الحب التضحية والعناء من أجل تربيته و تعليمي

This thesis is based on the following papers:

1- Agronomic performance of annual self-reseeding legumes and their self-establishment potential in Apuglia region in Italy

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2- Effect of annual self-reseeding legumes on vegetable subsequent crops in South of Italy. *In press*

3- Nitrogen fixation potential of annual self-reseeding legumes: comparative study between Viterbo and Bari. *In press*

Abstract

Soil fertility management is a major concern in organic farming. According to the EEC Regulation 2092/9, soil fertility must be maintained and/or increased by legumes cultivation. Native Mediterranean legumes ecotypes are more persistent than commercial varieties but their use in catch crop sequences is still limited. The N₂-fixing legumes crops as a local resource can be considered as an alternative to improve nitrogen nutrition and to increase crop yields. This study aimed to investigate the agronomic performance of seven annual self-reseeding legumes and the short-term effect of nitrogen supply on the subsequent crops. Results are presented from field experiment on sandy-clay soil, pH 8.1 and 1.6% of organic matter, in the south of Italy, where the self-reseeding legumes were evaluated during two cropping cycles for their adaptability and integration into Mediterranean cropping systems. Biological nitrogen fixation (BNF) was also performed using ¹⁵N isotopic dilution method.

For the first cropping cycle, *Trifolium* spp. performed better than *Medicago* spp. and of the seven tested species, five were more suitable to the site conditions. These species could be used for managing soil fertility and enhancing biodiversity in orchards. *Trifolium angustifolium* and *Medicago polymorpha* were the most performing species. They fixed 132 and 90 kg N ha⁻¹ of BNF, produced 1976 and 731 kg ha⁻¹ of seeds and 8.7 and 5.5 t ha⁻¹ of dry biomass (DM) respectively. In contrast, *Medicago radiata* and *M. rigidula* were the least performing species.

For the second cropping cycle, their self-establishment capacity and their effect as green manure on two subsequent crops of zucchini and lettuce were investigated. Results showed again that *Trifolium* spp. performed better than *Medicago*. Except for *M. radiata* and *M. rigidula*, regenerated species appear to sustain optimum level of soil-available nitrogen and induced positive effects on both zucchini and lettuce growth parameters. Zucchini and lettuce marketable yield significantly increased influenced by the preceding legumes, on average 53% and 24% respectively over the control. *T. angustifolium* was again the best performing and promising species producing the highest DM (7.74 t ha⁻¹) and fixing nitrogen (146.7 kg N ha⁻¹) symbiotically. It induced the best effect on all zucchini and lettuce parameters including crop yield (42.66 and 48 t ha⁻¹ respectively). In contrast, *M. polymorpha* was less performing (0.3 t ha⁻¹ of DM and 11.5 kg ha⁻¹ of BNF). Given the overall performance of all species, it was determined that *T. angustifolium* had the greatest potential for further development in this environment.

Key words: Annual self-reseeding legumes *Trifolium* spp., *Medicago* spp., biological nitrogen fixation, green manure, rotation, subsequent crops, soil fertility.

Riassunto

La gestione della fertilità del suolo è l'aspetto di maggiore interesse in agricoltura biologica. Secondo il Regolamento CEE 2092/91, la fertilità del suolo deve essere mantenuta e/o incrementata mediante la coltivazione delle leguminose. Gli ecotipi delle leguminose native del Mediterraneo sono più persistenti delle cultivars importate, ma il loro uso come "catch crops" è ancora limitato. Come risorse locali le leguminose fissatrici di azoto possono essere considerate una alternativa per migliorare la nutrizione azotata e aumentare la resa delle colture. Questa ricerca mira a valutare le prestazioni agronomiche di sette leguminose annuali autoriseminanti, e il loro impatto a breve termine sulla disponibilità di azoto per le colture in successione. Lo studio è stato condotto nel sud d'Italia, su terreno sabbioso-argilloso, con pH 8,1 e 1,6% di sostanza organica. Le leguminose sono state valutate per due cicli colturali per la loro adattabilità e integrazione nei sistemi colturali Mediterranei. L'azoto-fissazione simbiotica è stata determinata mediante la tecnica di diluizione dell'isotopo ^{15}N .

Durante il primo ciclo colturale, le specie di trifoglio sono risultate in media più adatte di quelle di medica. Tra le sette specie saggiate, cinque si sono rivelate più adatte alle condizioni pedo-climatiche del sito. Tali specie potranno essere impiegate per la gestione della fertilità del suolo e per incrementare la biodiversità nei campi coltivati. *Trifolium angustifolium* e *Medicago polymorpha* sono state più promettenti delle altre specie. Rispettivamente la resa in azoto fissato è stata di circa 132 e 90 kg N ha⁻¹, con una produzione di seme di 1976 e 731 kg ha⁻¹ e una produzione di biomassa secca di 8,7 e 5,5 t ha⁻¹. *Medicago radiata* e *M. rigidula* invece sono state le meno promettenti.

Nel secondo ciclo colturale, l'indagine ha interessato il potenziale di autorisemina ed il loro effetto come colture da sovescio su due colture in successione: zuccina e lattuga. I risultati hanno di nuovo dimostrato che le specie di trifoglio sono più idonee in confronto alle mediche. Eccetto *M. radiata* e *M. rigidula*, tutte le specie che hanno riseminato sembrano sostenere un ottimo livello di azoto disponibile nel suolo e indurre un effetto positivo su entrambi gli indicatori di crescita di zuccina e di lattuga. La resa delle due colture in successione è significativamente aumentata grazie all'influenza delle leguminose precedenti, in media, rispettivamente del 53% e del 24% rispetto al controllo. *T. angustifolium* è stato di nuovo il più promettente tra tutti. Ha prodotto la più alta quantità di biomassa secca (7,74 t ha⁻¹) ed ha fissato biologicamente una quantità di azoto di circa 146,7 kg ha⁻¹. Di conseguenza, è stato in grado di indurre un effetto positivo su tutti i parametri della zuccina e della lattuga, inclusa la resa (rispettivamente 42,66 e 48 t ha⁻¹). Al contrario *M. polymorpha* è stata la meno promettente (0,3 t ha⁻¹ di biomassa secca e 11,5 kg N ha⁻¹). Dei risultati ottenuti si può dire che *T. angustifolium* ha un grande potenziale per un ulteriore sviluppo in questo ambiente.

Parole chiave: Leguminose annuali autoriseminanti, *Trifolium* spp., *Medicago* spp., azoto fissazione simbiotica, concime verde, rotazione, colture in successione, fertilità del suolo.

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Introduction

Everyone in world depends completely on Earth's ecosystems and the services they provide (food, water, disease management, climate regulation, spiritual fulfilment, and aesthetic enjoyment). Over the past 50 years, humans have changed these ecosystems more rapidly and extensively than any comparable period of time in human history, largely to meet rapidly growing demands for food, fresh water, timber, fiber, and fuel. This has resulted in a substantial and largely irreversible loss in the diversity of life on Earth (Millennium Ecosystem Assessment, 2005).

The changes that have been made to ecosystems have contributed to substantial net gains in human well-being and economic development, but these gains have been achieved at growing costs in the form of the degradation of many ecosystems services, increased risks of nonlinear changes, and the exacerbation of poverty for some groups of people. These problems, unless addressed, will substantially diminish the benefits that future generations obtain from ecosystems (Millennium Ecosystem Assessment, 2005).

In agriculture world, after the increasing population in 1960s the green revolution lead to a strong use of mechanization, chemical-synthetic inputs and high yielding varieties leading within the following 4 decades to environmental problems (Dioxin, pollution, DDT...), overproduction with a saturation and liberalization of agro-food market and the use of genetically modified organisms.

Modern specialized agriculture systems carry out productive functions only by adding large auxiliary inputs (synthetic fertilizers, pesticides, etc) and paying little attention to environmental degradation and human health risks (Caporali and Onnis, 1991).

The doubling of agriculture food production during the past 35 years was associated with a 6.87-fold increase in nitrogen fertilization. The anticipated next doubling of global food production would be associated with approximately 3-fold increase in nitrogen fertilization rates. These projected changes would have dramatic impact on the diversity, composition and functioning of the remaining natural ecosystems of the world (Tilman, 1999). To correct these negative tendencies it is necessary to return to environmentally sound agriculture and to implement it in modern agroecosystem (Caporali *et al.*, 1989).

Consequently, in the last decade, environmental awareness leads a worldwide interest in organic agriculture (Schmid *et al.*, 1991 and Niggli and Lockeretz, 1996). The foundation of organic agriculture lies in the health of the soil. According to ECC Regulation 2092/91 (Annex1-A- 2.1), soil fertility, in the first instance, must be maintained by the cultivation of legume and green manuring.

Legumes make an important contribution to plant nutrition by fixing nitrogen and improving soil characteristics. Main streams of nitrogen flow are fixed in the long term by organizing and optimising the site-adapted crop rotation. It is considered one of the most important technological methods in organic agriculture to guarantee high and constant yields with the minimum reliance on external inputs (Köpke, 1996).

An alternative approach may be to manage legumes as green manure crops by cutting and incorporating them directly in the field (Stopes and Millington, 1995). Accumulation of organic matter and nitrogen in the soil, weed suppression and yield increase of the following crop are the most relevant benefits achieved by incorporating leguminous green manure within crop rotation (Caporali *et al.*, 2004).

Successful establishment of annual legumes is achieved only with the use of varieties that have both high persistence and high productivity within the specific environment in which they are used (Köpke, 1996; Rochon *et al.*, 2004). Fara *et al.* (1997) found that in the Mediterranean (Med.) areas native ecotypes were, on average, more persistent and better adapted than commercial varieties. (Ehrman and Cocks, 1990) also reported that local legumes plants resources are more likely to provide adapted pasture plants than imported cultivars.

Sheaffer *et al.*, (2001) reported that annual legumes (*Medicago and Trifolium spp*) have the potential to diversify crop rotations and to affect yields of a subsequent crop. Although they are well known forage crops in cereal-ley farming under the Mediterranean climate throughout the world, their use in cash crop sequences is virtually unknown. A lack of knowledge of annual self reseeding legumes ecology and physiology and their integration into cropping systems under Mediterranean conditions are the most important factors that may discourage farmers in Mediterranean region from adopting them (Caporali and Campiglia, 2001).

Ley-farming systems (including cereal and annual legumes) are used extensively in southern Australia. Annual legumes have replaced fallow in cereal agroecosystems. Self-regenerating pastures of annual legumes grown in rotation with wheat contribute to sustainable agriculture in the cereal/pasture (sheep) zone of southern Australia by providing organic N, conserving and building soil, and providing a break from cereal pests and diseases. This wheat/sheep system is practiced in an annual precipitation zone that occupies as much as 30 million hectares (Crawford, 1989)

The success of self-regenerating legumes in southern Australia has generated interest in other regions having Med. climate. Over the last thirty years, the annual legumes/cereals rotation has been evaluated in Algeria (Rapport IDGC, 1980), Tunisia (Mouaffak, 1976), Syria (ICARDA, 1982), Libya, Jordan (Chatterton and Chatterton, 1984), Iraq (Radaman *et al.*, 1978) and Morocco (Bounjemat, 1990). Despite considerable optimism and effort, establishment of ley-farming system has faced many failures (Bounjemat *et al.*, 1992) due to insufficient information on the ecology and physiology of medics/clovers, or lack of know-how on their importance. For instance, in both Algeria (Rapport IDGC, 1980) and Syria (Cocks and Ehrman, 1987), the cultivars

used failed because they were susceptible to frost as they were commercial Australian cultivars that were developed for warm winter Med. type climate and acid soil of southern Australia. However, further researches are still needed in order to re-evaluate the role of legumes in the cropping system and to select cultivars adapted to Med. conditions.

The Mediterranean Agronomic Institute of Bari (IAMB) has been engaged in the last five years in testing different legume species (Al-Bitar, 2005). Fifteen Australian cultivars were tested, five of them showed to be promising but it was not possible to proceed with their evaluation (not available in the market). Interest was moved towards other species from the International Center for Agricultural Research in the Dry Areas (ICARDA, Aleppo-Syria) collected in Mediterranean area.

The present study is a boarder programme of a three year investigation (2005-2007) aimed at the screening of seven native species of annual self-reseeding legumes (Medics and Clovers) for the purposes of exploiting their agronomic performance and discussing their integration into sustainable Mediterranean cropping systems. It was conducted according to the following four hypotheses: i) the annual legumes are flexible compounds within cropping systems; ii) the nitrogen fixed by legumes contribute to enrich the soil in nitrogen; iii) the precrop effect increase the nitrogen nutrition and the yield of subsequent crops; iv) they can influence in medium and long-term physical, chemical and biological soil properties.

In order to verify those hypotheses, the research study attempts in the first year to study the agronomic performance of the selected annual species (Medics and Clovers) under Apulia region conditions, to quantify their ability to fix nitrogen (^{15}N isotope dilution method) and to select the most suitable species for their integration into Mediterranean organic cropping systems.

The second year study aims at evaluating their self-establishment capacity, investigating their effect on soil nitrogen enrichment, evaluating their ability to fix atmospheric nitrogen and studying their impact on subsequent crops (zucchini and lettuce).

So the research questions are: which of those species are better adapted to perform under the Mediterranean climate? And which are best when integrated into rotation programme?

Chapter 1: State of knowledge

1.1. Mediterranean climate

Mediterranean climate is a climate that resembles those of the lands bordering the Mediterranean Sea. These climates generally occur on the western coasts of continental landmasses, roughly between the latitudes of 30° and 45° north and south of the equator.

The region around the Mediterranean Sea has a climate of mild rainy winters and hot dry summers. This distinctive climate pattern is also found in four other widely separated parts of the world: California, Chile, South Africa and Australia. In total, these five areas comprise only about 2% of the earth's landmass. The largest of them is the Mediterranean itself, about 60% of the world's Mediterranean climate, followed by South and Western Australia, which together equal about 22%. The remaining three, from largest to smallest respectively: California (10%), Chile (5%), and South Africa (3%) (Dallman, 1988).

At least 65% of the year's precipitation occurs in winter. Annual precipitation ranges between 275 and 900 mm, the average temperature in winter months is below 15°C and the hours per year at which the temperature falls below freezing do not exceed 3% of the total.

Plants developing in such climates are periodically subjected to a combination of stresses including not only the lack of water and high temperature coupled to high evaporative demand and high light intensity in summer, but also limitation in the content of nitrogen, phosphorus and other nutrients in the soil. Cold winter is also relevant in some areas. This situation is sometimes aggravated by salinity, fire, overgrazing, etc (Sanchez-Diaz, 2001).

The Mediterranean Basin shows a high plant-species diversity. It has also been a centre of origin of numerous cultivated species. Undeniably, the Mediterranean Basin has influenced all the other Mediterranean-climate regions, through the introduction of typical crop and domestic animal breeds and through the dispersion of Mediterranean grasses and weeds (Sanchez-Diaz, 2001).

1.2. Soil overview

Soil is a layer of organic and mineral substances covering most of the Earth's surfaces. It is created by a slow and constant physical and chemical breakdown of rock and the action and turnover of living organisms. In fact, it takes approximately five hundreds years to replace one inch of soil by these processes, making it critical to keep the soil fertile and productive as well as to prevent its erosion (Vantine and Verlinden, 2003).

Minerals comprise 45% of the soil, water and air 50% and organic matter 2% to 5%. A soil high in organic matter and humus is characterized as having good tilth or soil structure. This type of soil is easy to work and is in overall good health, which encourages the processes necessary to produce a healthy plant.

The foundation of organic farming lies in the health of the soil, a fertile soil provides essential nutrients to the growing plants and helps support a diverse and active biotic community (Vantine and Verlinden, 2003).

1.3. Nitrogen

1.3.1. Role of nitrogen in the biosphere

All organisms require nitrogen to live and grow. Nitrogen is an essential component of DNA, RNA and proteins the building block of life (Harrison, 2003). There is a huge reservoir of nitrogen in the atmosphere, approximately 80% of the air consists of nitrogen gas (N_2) in the form of a colourless, odourless and non-toxic gas (Silva and Uchida, 2000). The same nitrogen gas found in the atmosphere can be found in spaces between soil particles. However, plants are unable to use this form of nitrogen. Certain microorganisms found in the soil are able to convert atmospheric nitrogen into forms that plants can use (Killpack and Buchholz, 1993).

Nitrogen is an essential plant nutrient. It is the nutrient that is most commonly deficient in soil and contributing to reduce agricultural yields throughout the world (Montanez, 2000). Nitrogen is required by plants in higher concentrations than any of the other elements. Plants grown without nitrogen grow very slowly, stunted and show a general yellowing of the foliage. The symptoms of nitrogen deficiency will first occur in the older leaves. The leaves turn from a pale green colour to yellow and eventually leaf drop occurs. The younger leaves remain green for longer periods of time but eventually, the whole plant exhibits a pale green to yellow colour (Beardsell, 1995). So, nitrogen is often the limiting factor for growth and biomass production in all environments when there is suitable climate and availability of water to support life (Deacon, 2001).

1.3.2. Nitrogen cycle

The nitrogen cycle is one of the major biogeochemical cycles, it is a continuous series of natural processes by which nitrogen passes from the air to the soil, plants and ultimately to sustain all animal life and then returns back to the air or soil through decay or denitrification. The nitrogen cycle consists of various storage pools of nitrogen and processes by which the pools exchange nitrogen.

In order for nitrogen to be used by plants it must be fixed in the form of ammonium ions (NH_4^+) and the ion nitrate (NO_3^-). Most plants obtain the nitrogen they need as inorganic nitrate from the soil which is quickly incorporated into protein and other organic nitrogen compounds (Harrison, 2003). Decomposers, found in the upper soil layer, break down protein in dead organisms releasing ammonium ions which can be converted to

other nitrogen compound. This process is known as mineralization (Burdass, 2005). Then in nitrification process, nitrogen compounds are oxidized to nitrite and nitrate. Finally, nitrite and nitrate are converted to dinitrogen (N_2) in the processes called denitrification (Harrison, 2003).

1.3.2.1. Nitrogen fixation

Most of the reactive nitrogen produced by humans comes from manufacturing nitrogen for synthetic fertilizers and industrial use. Reactive nitrogen is also created as a by-product of fossil fuel combustion and by some (nitrogen-fixing) crops and trees in agroecosystems (Millennium Ecosystem Assessment, 2005)

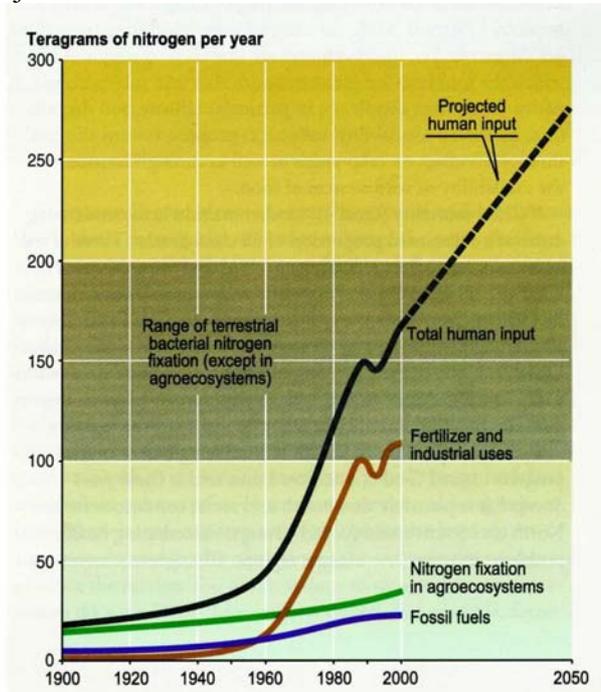
According to Smil (2000) there are four ways to convert N_2 into a form readily available to plants and, hence, to animals and humans:

- Biological fixation: some symbiotic bacteria (most often associated with leguminous plants) and some free-living bacteria are able to fix nitrogen and assimilate it as organic nitrogen. An example of mutualistic nitrogen fixing bacteria is the *Rhizobium* bacteria which live in plant root nodules.
- Industrial fixation: N_2 is converted together with hydrogen gas (H_2) into ammonia (NH_3) fertilizer .
- Combustion of fossil fuel automobile engines and thermal power plants which release NO_x .
- Other processes like the formation of NO from N_2 and O_2 due to photons and lightning which are important for atmospheric chemistry but not for terrestrial or aquatic nitrogen turnover (Smil, 2000).

Human activities increase nitrogen deposition in a variety of ways including burning of both fossil fuels and forests, fertilizing crops with nitrogen-based fertilizers, ranching during which livestock wastes release ammonia into the soil and allowing sewage and septic tanks to leach into streams, rivers and groundwater (Gordon, 2005).

The global trends of anthropogenic N fixation in relation to natural one is shown in figure 1.

Figure 1: Global trends in the creation of reactive nitrogen on Earth by human activity, with projection to 2050



Source: (Galloway *et al.*, 2004)

A- Symbiotic nitrogen fixation

Symbiotic nitrogen fixation is a mutual relationship between the plant and a microbe. The microbe firstly invades the root and later forms nodules in which nitrogen fixation takes place (Willson, 2005). Many members of legume plant family have the special ability to use symbiotic nitrogen fixation to meet their N needs (Silva and Uchida, 2000).

Legume plant and seed tissue are relatively high in protein. This can be directly attributed to a legumes ability to supply most of its own nitrogen needs with the help of symbiotic *Rhizobia* bacteria living in their roots (Donahue, 1998). The plant-bacteria symbiosis is initiated when the bacterium detects a chemical signal exuding naturally from the roots of the legume (Fox *et al.*, 2001).

A recent study has estimated that 10 to 20 percent of annual nitrogen input to soil in the United States comes from symbiotic nitrogen fixation. In developing countries where nitrogen is not readily available, this benefit is even more important (Lamond *et al.*, 1988).

Nitrogen fertilizers are expensive inputs for crop production. Their manufacturing, packaging, transport and application are extremely energy consuming (Silva and Uchida,

2000). 20 - 30% of the energy costs of plant production are associated with the generation and application of nitrogen fertilizer which equals to \$7 to 10 billion annually, whereas even modest use of alfalfa in rotation with corn could save farmers in the U.S. \$200 to 300 million (Peterson and Russelle, 1991). The cultivation of legumes allows the saving of approximately the consumption of 0.2 tons of fuel per hectare that corresponds to the production of 600 kg of CO₂ per hectare.

Additionally, nitrogen fertilizers affect the balance of the global nitrogen cycle and may pollute groundwater, increase the risk of chemical spills and increase atmospheric nitrous oxide (Montanez, 2000). Conversely, symbiotic nitrogen fixation by legumes results in a decrease in the consumption of fossil fuel energy and therefore decreases the agricultural contribution to global warming. Additionally, it avoids problems of contamination of water resources resulted from leaching and runoff of excess fertilizer (Silva and Uchida, 2000).

In conclusion, the process of symbiotic nitrogen fixation offers an economically attractive and ecologically sound means of reducing external nitrogen input and improving the quality and quantity of internal resources (Montanez, 2000; Silva and Uchida, 2000). Nitrogen fertilizer is frequently unavailable to subsistence farmers, leaving them dependent on N₂ fixation by legumes or other N₂-fixing organisms. Legumes are thus a pillar in the development of sustainable agricultural systems (Graham and Vance, 2003).

B- Factors affecting the amount of nitrogen fixed

According to Mulongoy (1995), factors that limit biological nitrogen fixation are divided into edaphic, climatic and biotic factors. Regarding edaphic factors, excessive moisture, drought, soil acidity, phosphorus deficiency and excess of Ca, Mo, Co and B are among the most important factors. Excessive moisture prevents the development of root hair and sites of nodulation, while drought reduces the number of *Rhizobia* in soils, and inhibits nodulation and nitrogen fixation. The two important climatic determinants affecting BNF are temperature and light, extreme temperature affects fixation adversely by affecting the enzymatic process while the availability of light regulates photosynthesis upon which nitrogen fixation depends. Among biotic factors, the absence of the required *Rhizobia* species constitute the major constraint in the nitrogen fixation process.

C- Methods used for estimating the amount of nitrogen fixed

According to Loges *et al* (2000), the amount of nitrogen fixed by legumes is often difficult to quantify, as it is difficult to distinguish how much is taken up from the atmosphere and how much is taken from the soil and, subsequently, returned to it again. Other sources of confusion may also stem from the addition of nitrogen to the soil by rain and the mineralization of organic matter exists already in the soil.

Various methods have been used to provide estimates of N₂ fixed in forages. Nodules number, weight and plant dry weight are among the earliest, most inexpensive and

simplest methods (Bowren *et al.*, 1969; Bell and Nutman, 1971; Heichel and Vance, 1979; Heichel *et al.*, 1984). But they do not give a measure of how much N₂ is fixed. Reliable methods are needed to quantify biologically fixed nitrogen in legumes to exploit this natural inexpensive source of nitrogen (Danso, 1985).

The total nitrogen difference between a fixing and a non-fixing crop has been extensively used to estimate N₂ fixed in various legumes (Bell and Nutman, 1971; LaRue and Patterson, 1981; Phillips *et al.*, 1983). The non-fixing crop is used to assess the amount or portion of the fixing crop's total nitrogen that came from soil. The assumption, therefore, is that the two crops, which may even belong to vastly different species, take up similar amounts of soil nitrogen. As pointed out by Rennie and Rennie (1983) and Danso (1985), the validity of this assumption is doubtful in most cases. The method also lack precision. Moreover, when soil or fertilizer nitrogen levels are relatively high, N₂ fixation may be low enough to be obscured by sampling, analytical and other experimental errors (Danso *et al.*, 1985). The most widely used methods are the acetylene reduction technique and the ¹⁵N methodology (Papastylianou, 1999).

The acetylene reduction technique provides an instant measure of nitrogenase activity (but not necessarily of N₂ fixed) under the experimental conditions (Mulongoy, 1995). Although simple, inexpensive and sensitive, this technique suffers from serious drawbacks, particularly since it is only a short-duration enzyme activity assay which does not take into account the existence of diurnal and seasonal variations in nitrogen fixation thus, makes extrapolation of such instantaneous assays to total nitrogen fixed over a growing season questionable (Ayanaba and Lawson, 1977; De-Polli *et al.*, 1977; Fried *et al.*, 1983).

The most reliable of the techniques currently available for measuring N₂ fixation are based on the use of nitrogen stable isotopes (Unkovich and Pate, 2001). However, by using the differences that exist in ¹⁵N enrichment of soil and the atmosphere (natural ¹⁵N-abundance technique), or by artificially inducing such a difference through the addition of ¹⁵N-enriched or depleted fertilizers to soil (in several reports called ¹⁵N-isotope-dilution method), it has been possible in the field to estimate directly the proportion or amount of nitrogen in a plant that is derived from symbiotic N₂ fixation (Kohl and Shearer, 1981; Bergersen and Turner, 1983; Heichel *et al.*, 1985). According to Chalk (1985), more than 50 research papers on N₂ fixation involving the addition of ¹⁵N enriched and depleted materials to soil have been published since 1975. Much of this interest in the technique is attributable to its applicability in the field and its ability to give an integrated estimate of N₂ fixed over one or several growing seasons. The lowered cost of ¹⁵N-labelled fertilizers and the availability of cheaper instruments for ¹⁵N/¹⁴N-ratio analysis have also undoubtedly made a significant contribution (Danso *et al.*, 1985).

A great advantage with the use of ¹⁵N isotope dilution technique is that it is the only method that can distinguish between soil, fertilizer and fixed nitrogen in field-grown

crops. Estimates made using the isotope dilution technique have established that most forage legumes derive a large proportion of their nitrogen from fixing atmospheric N₂ and, in general, this is in excess of 70 - 80 % of their total N requirements (Danso *et al.*, 1985).

The determination of the level of nitrogen fixation by the ¹⁵N isotope dilution technique requires an assessment of the ¹⁵N/¹⁴N ratio in soil, the lower the ¹⁵N/¹⁴N ratio, the better and the fixer it is. This assessment is made by selecting an appropriate reference crop. Errors associated with using poor reference crops may be high at low values of fixation but become negligible at high values of fixation. With such a high proportion of the nitrogen in forage legumes generally coming from fixation, the effect of a poor choice of a reference crop on N₂ fixation estimate is low. Only a quantitative determination of the magnitude of the effect requires the use of a suitable reference crop. The criteria that need to be observed in selecting suitable reference crops include: lack of N₂-fixing ability, nitrogen uptake patterns of the legume and the reference crop should match closely, similar periods for planting and harvesting and proper sampling procedures (Danso *et al.*, 1985).

1.4. Legumes

1.4.1. Taxonomy of legumes

Traditionally, the legume family has been divided into three subfamilies: Caesalpinieae, Mimosoideae and Papilionoideae (ILDIS, 2005). The majority of the Caesalpinieae and Mimosaceae are tropical or subtropical trees and shrubs. Papilionoideae, the largest of the three subfamilies with 476 genera and 13,860 species, is also the most diverse and widely distributed and includes most of the familiar domesticated food and forage crops and model genetic species.

1.4.2. Importance of legumes

With some 20,000 species, the legumes are the third largest family of higher plants. The Leguminosae are second to cereal crops in agricultural importance based on area harvested and total production.

Grain and forage legumes are grown on some 180 million ha or 12% to 15% of the Earth's arable surface (Table 1). They account for 27% of the world's primary crop production, with grain legumes alone contributing 33% of the dietary protein nitrogen needs of humans (Vance *et al.*, 2000). Seeds of grain legumes contain at least 20% to 40% of protein. In many places of the world, legumes complement cereals or root crops, the primary source of carbohydrates, in terms of amino acid composition (Gepts *et al.*, 2005).

Table 1: Major crops production and area harvested

Crop	Production (kg .10⁹)	Area Harvested (ha.10⁶)
Grain legumes	275	160
Forage legumes	605	20
Wheat	583	214
Rice (<i>Oryza sativa</i>)	590	152
Maize (<i>Zea mays</i>)	609	138
Barley (<i>Hordeum vulgare</i>)	141	54
Potatoes (<i>Solanum tuberosum</i>)	308	19
Cassava (<i>Manihot esculenta</i> Crantz.)	179	20
Total	3,320	777

Source: (FAO, 2005a)

Moreover, grain legumes provide about one-third of processed vegetable oil which is more than 35% of the world's processed vegetable oil for human consumption (Graham and Vance, 2003). Legumes, also, provide essential minerals required by humans and produce health promoting secondary compounds that can protect against human cancers and other minerals that protect the plant against the onslaught of pathogens and pests (Dixon *et al.*, 2002; Ndakidemi and Dakora, 2003). On the other hand, an efficient animal based agriculture is sustained by legumes since they provide high quality forage.

In addition to traditional food and forage uses, legumes can be milled into flour used to make bread, doughnuts, tortillas, chips, spreads, and extruded snacks or used in liquid form to produce milks, yogurt and infant formula (Garcia *et al.*, 1998). A hallmark trait of legumes is their ability to develop root nodules and to fix N₂ in symbiosis with compatible *Rhizobia*. Some 40000 to 60000 million kilograms of N₂ are fixed by agriculturally important legumes annually with another 3000 to 5000 million kilograms fixed by legumes in natural ecosystems (Smil, 1999).

1.4.3. Annual self-reseeding legumes

1.4.3.1. Annual self-reseeding legumes physiology

A- Seed dormancy

Seed dormancy is when seed does not germinate even though all the necessary environmental conditions for growth are satisfied. Despite the fact that various mechanisms and components of seed dormancy have been described in legumes, hardseedness, embryo dormancy and high-temperature dormancy are probably the most important ones in Mediterranean legumes.

Hardseedness, or impermeability of the seed coat to water, prevents imbibitions by the seed coat and other enclosing tissue such as endosperm and pericarp. This develops during seed maturation and is under both genetic and environmental control. The embryos of such seeds will germinate readily by the influence of diurnal fluctuating temperatures and other natural or artificial processes that scarify the seed coat to produce soft seed in the presence of water and oxygen once the seed coat and other surrounding tissues are removed or damaged. This is the case of the legumes (*Trifolium* and *Medicago spp.*) (Jansen and Ison, 1995; Taiz and Zeiger, 2002). Newly ripened seeds of annual legumes have high levels of hardseedness, but the rate of seed softening under field conditions differs greatly among accessions and between species (Del Pozo and Aronson, 2000).

Embryo dormancy refers to a dormancy that is inherent in the embryo and is not due to any influence of the seed coat or other surrounding tissues (Taiz and Zeiger, 2002).

High-temperature dormancy has been detected in various species of *Trifolium* (Jansen and Ison, 1995; Norman *et al.*, 1998). This is an enforced dormancy that occurs when seeds are exposed to temperatures of 30-40 °C. In this range of high temperatures, seeds imbibe water but fail to germinate, even though the same seeds germinate when subsequently placed at 15 °C. It has been suggested that this type of seed dormancy could play a role in preventing seeds from germinating in the case of false break (Jansen and Ison, 1994).

B- Seed germination

Once seed dormancy has been broken down and soil has enough moisture, the percentage and rate of germination depends largely on temperature. The relationship between seed germination and temperature has been studied for various annual legume species (Covell *et al.*, 1986 and Durmur *et al.*, 1990).

Merou and Papanastasis (2000) reported that every annual legume species has its own germination pattern as the various environmental conditions affect seeds differently.

For Mediterranean legumes seeds germination, optimum temperature ranges from 16 to 22 C, whereas maximum temperature ranges from 30 to 40 °C (Covell *et al.*, 1986).

During regeneration, the presence on the soil surface of plant residues, including stubble, disturbs legume seed germination. Also the erect growth habit and high density of grasses limits the development of legume seedlings (Rochon *et al.*, 2004).

C- Flowering

Annual legumes show both ecotypic differentiation and a high degree of plasticity in flowering time. Although severe water deficits may affect the development of annual legumes, time to flowering is mainly influenced by genotype, temperature and photoperiod (Roberts *et al.*, 1997).

D- Growth

Legumes usually have higher nitrogen concentration in both vegetative and reproductive organs than grasses and other dicotyledonous plants (Del Pozo, 1992 and McKey, 1994). According to McKey (1994), nitrogen-rich leaves of legumes should allow higher rates of photosynthesis especially at high light intensities and, therefore, favor rapid growth of leaves and plant production during favorable periods.

1.4.3.2. Annual legumes in Mediterranean region

A- Diversity and abundance

Being an annual has many advantages, especially in the Mediterranean Basin itself where annuals may be considered something of a regional specialty (Figure 2) , often constituting half of the regional vegetation whereas they rarely amount to more than one-tenth in other parts of the world (Raven, 1973; Blondel and Aronson, 1999).

Among Mediterranean annual invaders, only one group is normally greeted with any enthusiasm: the annual, nitrogen-fixing legumes of high pastoral value such as *Medicago*, *Trifolium* and many others in addition to various suites of adaptations allowing survival during periods of prolonged drought, for example, these annual legumes also have remarkable capacity to ‘capitalize’ rapidly on short, intense periods of resource availability and ,thereby, increase their number of offspring dramatically under such conditions (Del Pozo and Aronson, 2000). In fact, annual medics and clovers are considered to be the most important winter annual forage species for rotations in the Mediterranean region (Sheaffer and lake, 1997).

Despite their numbers, annual Mediterranean legume species can conveniently be considered as a single functional group in fields or pastures, i.e., a group of species with similar responses to a given factor, and having a set of common biological and physiological attributes that correlate with their overall performance and fitness (Lavorel *et al.*, 1998). However, they do of course differ in many ways, including their responses

to a given disturbance or stress, among genera, species and even accessions of a given species (Del Pozo and Aronson, 2000).

B- Development and adaptation to Mediterranean seasonal unpredictability

In Mediterranean region, plants are exposed to various stresses as the result of interactions with abiotic factors like temperature (especially low and high extremes), excess or insufficient soil water availability (i.e., temporary water logging and prolonged drought), soil acidity or salinity and nutrient deficiencies. Biotic factors also play a major role in plant ecology and evolution in these regions including plant-plant interactions (i.e., intra and interspecific competition, neighbor relationships, shading), herbivore impact (i.e., insect defoliation, overgrazing) and mutualism or other kinds of interactions with fungi, bacteria and other micro-organisms. Like all other plants and animals in the Mediterranean, annual plants must also cope with highly seasonal environments and a remarkable degree of climatic unpredictability (Del Pozo and Aronson, 2000).

Annual self reseeding legumes may have valuable traits adapted to Mediterranean region that can be exploited in cash crop sequences as they (a) grow during the cool season (b) die in the early summer (c) regenerate after fall rains providing cover that can be used as either green manure or a dry mulch for the succeeding crop (d) tolerate shade (e) provide weed control through good growth coverage (f) provide significant quantities of fixed nitrogen while conserving soil and water resources and sustaining or improving soil productivity and (g) allow the use of minimum tillage or no till practices. Because of these characteristics and advantages, annual legumes might be used for improving the agroecological performance of cash crop sequences such as two year rotation (Caporali and Campiglia, 2001).

In the Mediterranean region, annual legumes tend to grow and reproduce during the cool and wet seasons only. In addition to rainfall unpredictability, the length of the growing season is also very variable. Annual legumes show ecotypic differentiation in flowering time in more or less close correlation to the aridity of the collection site (Ehrman and Cocks, 1996; Piano and Talamucci, 1996). It has frequently been demonstrated that flowering time plasticity is a common feature of adaptive significance for annuals, including legumes, in arid or semi-arid environment including Mediterranean region (Fox, 1989, 1990; Ehrman and Cocks, 1996).

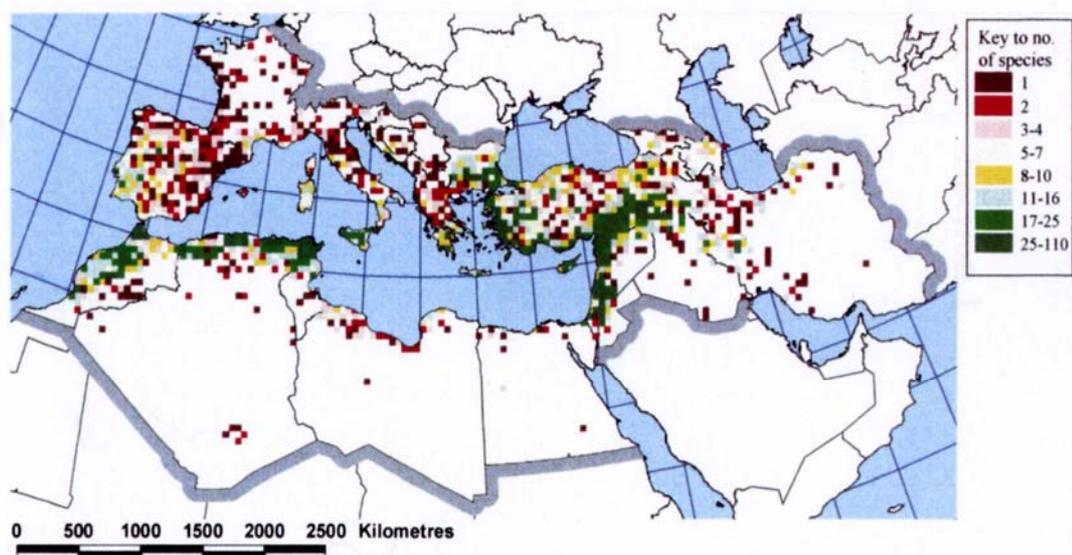
Also the occurrence of summer and early autumn rain in Mediterranean region, which result in false breaks of season, i.e. an early germination of seeds which then die in the absence of follow-up rains, is another important environmental constrain for annual plants. To cope this, annual legumes show high levels of seed dormancy and mechanism that regulate the pattern of seed germination over more than one growing season. Seed survival in the seed bank is also of great importance to populations, predation by ants and other animals and fungal attack (Del Pozo and Aronson, 2000).

Therefore, the adaptation and long-term persistence of annual legumes in fluctuating environment of Mediterranean region depend largely on the capacity of plants to complete their reproductive cycle in order to ensure seed production and on the survival of seeds in the soil over several seasons in the seed-bank (Del Pozo and Aronson, 2000; Hekneby *et al.*, 2001).

Moreover, softening of hard seeds in the autumn rather than earlier in the summer months is a desirable characteristic for reducing seed losses from the breaks of season in annual pasture legumes in Mediterranean environment (Taylor, 1996). For instance, in *Medicago polymorpha* where the hard seed breakdown occurs between early August and September, the proportion of hard seeds remains higher than 85% (Rochon *et al.*, 2004).

So as result of the previous reasons, the annual self-regenerating legumes are extraordinarily suited to Mediterranean-type climates (Fox, 1989, 1990; Ehrman and Cocks, 1996; Piano and Talamucci, 1996).

Figure 2: Distribution of collecting sites of 48082 accessions of annual legumes from Mediterranean countries.



Source: Nigel and Bennett (2001)

C- Challenges

The main challenge with sustaining well-established annual legume system in Mediterranean region can arise from that soil in most Mediterranean region are low in phosphorus which legumes have a relatively high requirement for (Rochon *et al.*, 2004). Phosphorus is needed for plant growth, nodule formation and development and ATP synthesis (the energy source for bacteria), each process being vital for nitrogen fixation (Mulongoy, 1995). Moreover, deficiencies in calcium and trace elements can seriously limit nitrogen fixation through direct adverse effect on plant growth (Rochon *et al.*, 2004).

1.4.4. Soil benefits from using legumes

1.4.4.1. Soil organic matter

Because most crop residues contain much more carbon than nitrogen and bacteria in the soil need both, the nitrogen supplied by legumes facilitates the decomposition of crop residues in the soil and their conversion to soil building organic matter (Donahue, 1998).

1.4.4.2. Soil porosity

Several legumes have aggressive taproots reaching 1.83 to 2.43 m deep and 1.25 cm in diameter that open pathways deep into the soil. Nitrogen-rich legume residues encourage earthworms and the burrows they create. The root channels and earthworm burrows increase soil porosity and promoting air movement and water percolation deep into the soil (Donahue, 1998).

1.4.4.3. Recycle nutrients

Because perennial and biennial legumes roots penetrate deeply in the soil, they have the ability to recycle crop nutrients that are deep in the soil profile. This results in preventing nutrients (particularly nitrate) from being lost due to leaching below the root zone of shallower-rooted crops in the rotation (Donahue, 1998).

1.4.4.4- Improve soil structure

Research in both the United States and Canada indicated improved soil physical properties following legumes. The improvements are attributed to increases in more stable soil aggregates. The protein, glomalin, symbiotically along the roots of legumes and other plants serves as glue that binds soil together into stable aggregates. This aggregate stability increases pore space and tilth, reducing both soil erodibility and crusting (Donahue, 1998; Gaskell *et al.*, 2006).

According to research at Purdue University, fields with a winter cover crop that were used in the spring had 55% less water runoff and 50% less soil loss than fields without a winter cover (Jost, 1998).

1.4.4.5. Lower soil pH

Because inoculated, nodulated legumes acquire their nitrogen from the air as diatomic nitrogen rather than from the soil as nitrate, their net effect is to lower the pH of the soil. In greenhouse studies, alfalfa and soybeans lowered the pH in a Nicollet clay loam soil by one whole pH unit (Donahue, 1998).

1.4.4.6. Biological diversity

Legumes contribute to an increased diversity of soil flora and fauna lending a greater stability to the total life of the soil. They foster production of a greater total biomass in the soil by providing additional nitrogen. Soil microbes use the increased nitrogen to break down carbon-rich residues of crops like wheat or corn (Donahue, 1998).

1.4.4.7. Break pest cycles and weed suppression

Legumes provide an excellent break in a crop rotation that reduces the build-up of grassy weed problems, insects and diseases (Donahue, 1998; Mitchell *et al.*, 2000). For example, western corn rootworm larvae cannot tolerate the rotation with alfalfa so, this rotation can be an effective way to control this pest in corn (Roth, 1996). Legume rotation systems also help in managing weeds problems as shattercane, Johnson grass and perennial broad-leaved weeds (Lamond *et al.*, 1988; Reeves, 1994).

Currently, there is an interest in annual species of *Medicago* (annual medics) and other annual legumes for use as cover crops in grain cropping systems (Lesins and lesins, 1979). Crimson clover (*Trifolium incarnatum* L.) and subterranean clover (*Trifolium subterraneum* L.) have been shown to reduce weed density and dry weight of early season weeds (Johnson *et al.*, 1993; Teasdale, 1996).

1.4.5. Uses of legumes in organic agriculture

1.4.5.1. Cover crops

A cover crop is numerous numbers of plants, usually specific annual legumes growing and covering the soil surface (Whiting *et al.*, 2003). Winter annual legumes have been used as cover crops for centuries (Semple, 1928), it could be considered the backbone of any annual cropping system that seeks to be sustainable. Cover crops planted usually in late summer or fall to provide soil cover during the winter. Often a legume including for example clovers, vetches, medics and field peas is chosen for the added benefit of nitrogen fixation that improve the long-term nitrogen reservoir of the soil (Sullivan, 2003).

1.4.5.2. Forage crops

Forage is defined as herbaceous plant or plant parts fed to domestic animals. Generally the term refers to such material as pasturage, hay, silage and green chop, In contrast to less digestible material known as roughage. Short-rotation forage crops function both as cover crops when they occupy land for pasturage or haying, and as green manures when they are eventually incorporated or killed for no-till mulch. Examples include legume sods of alfalfa, sweet clover, trefoil, red clover and white clover, as well as grass-legume sods like fescue-clover pastures. For maximum soil-improving benefits, the forage should not be grazed or cut for hay during its last growth period, to allow time for biomass to accumulate prior to killing (Sullivan, 2003).

1.4.5.3. Living mulch

Living mulch is a cover crop that is interplanted with an annual or perennial cash crop. Living mulches suppress weeds, reduce soil erosion, enhance soil fertility and improve water infiltration. Examples of living mulches in annual cropping systems include overseeding hairy vetch into corn at the last cultivation, no-till planting of vegetables into subclover, sweetclover drilled into small grains and annual ryegrass broadcast into vegetables. Living mulches in perennial cropping systems are simply the grasses or legumes planted in the alleyways between rows in orchards, vineyards, Christmas trees, berries, windbreaks and field nursery trees to control erosion and provide traction (Sullivan, 2003).

1.4.5.4. Green manure

Growing green manure as a part of a crop rotation is an important part of organic farming systems. Green manures are plants grown to accumulate nutrients for the main crop, when they have built up maximum biomass, they are dug into the soil and at this point, they release nutrients and become fully decomposed within a short period of time (Eyhorn *et al.*, 2004).

An ideal plow down time for legumes is between early bud and early bloom. If they were allowed to reach flowering, there would be an increase in the amount of carbon which will slow down the availability of nitrogen for the next crop (Jost, 1998). They become too tough and will be more difficult to dig in. Moreover, soil organisms will find it difficult to break down and decompose (HDRA, 1998).

The green manure must be ready to dig in before the next crop is sown. There should not be a long gap between digging in the green manure and planting the next crop. This is to prevent nutrients from the green manure leaching out of the soil before being taken up by the next crop (HDRA, 1998). The time gap should not be longer than 2 to 3 weeks and green manure should not be ploughed deeply into the soil but only worked into the surface soil (Eyhorn *et al.*, 2004).

In fact, green manures are cheap alternatives to artificial fertilizers and can be used to complement animal manures. Generally they are important, especially on farms where there is not enough animal manure available and when it is not possible to bring in natural fertilizers from elsewhere (HDRA, 1998).

Legumes applied as green manure remain an important source of nitrogen in many crops production systems (Janzen and Radder, 1989; Becker *et al.*, 1995), winter annual legumes have been used as green manure for centuries (Semple, 1928). One fact to be considered is that benefits of green manures occur over the long term and are not always visible immediately (Eyhorn *et al.*, 2004). They do not only serve as a direct source of plant available nitrogen, but also have a great potential for increasing the availability of soil nitrogen to subsequent crop plants and for conserving nitrogen and enhancing the long term soil fertility (Ashraf *et al.*, 2004), Legumes plant material also known to increase the mineralization of soil native nitrogen which might be an important mechanism through which green manures enhance soil productivity (Fox *et al.*, 1990).

1.4.5.5. Crop rotation

A simple definition of crop rotation is the planting of different crops in recurring succession in the same field. Annual forage legumes can be important components of sustainable crop rotations in organic production systems (Heichel and Barnes, 1984; Hesterman, 1988).

The use of rotations associating cereals and legumes seems to be as old as agriculture. During Pharaonic era, in the Nile delta, rotations involved in addition to wheat and barley, grain and forage legumes. It had already been observed that cereals were exhausting for soil fertility whereas legumes were enriching.

1.4.6. Effect of leguminous rotation on subsequent crop production

For more than 2000 years, farmers have known that growing legume crops in rotation with cereals increases the growth and yield of the cereal crops by the double advantage of growing the legume with little or no additional nitrogen fertilizer, plus a nitrogen credit for the subsequent non-legume crop in which the yield can be significantly increased (Lamond *et al.*, 1988).

Waskom (1994) reported that plowing down a full stand of alfalfa will release as much as 110 kg ha⁻¹ of nitrogen in the first year after plow down. Moreover, a minimum of 33 kg ha⁻¹ of nitrogen should be credited in the first year after any legume crop.

So legume crops can be a very significant source of subsequent plant available nitrogen. Table 2 shows the nitrogen credit from legumes compared with other sources.

Table 2: Nitrogen credits for crop requirements

N Source	N Credit
Soil organic matter	13.6 kg N/% OM
Residual soil nitrate	1.6 kg N/ppm NO ₃ -N
Manure	4.5 kg N/ton dry manure
Irrigation water	1.2 kg N/AF x ppm NO ₃ -N
Previous alfalfa/sweet clover	55.0 kg N.ha ⁻¹
Other previous legume crop	33.0 kg N.ha ⁻¹

Source: Waskom (1994)

Nitrogen credits vary with the legume crop and its condition. Table 3 summarizes the nitrogen credit from legumes in crop rotations (Lamond *et al.*, 1988).

Table 3: Nitrogen credits from legumes in rotations

Legume crop		Nitrogen credit(Kg ha ⁻¹)
Alfalfa	> 80 % stand	110 – 154
	60 – 80 % stand	66 – 110
	< 60 % stand	0 – 66
Second year after alfalfa : half of first year		
Sweet clover		110 – 132
Red clover		44 – 88
soybeans		33 – 66

Source: Lamond *et al.*, (1988).

In recent study done on corn soybeans rotation, corn yield following soybeans was 5 to 20 percent more than continuous corn on the same farm (Roth, 1996). Moreover, Burket *et al.*, (2003) reported that broccoli grown with no nitrogen fertilizer after legume containing winter cover crops had a 50% higher average yield than that of fallow or cereal rye plots. Thus, the use of legumes cover crops showed that nitrogen fertilizer for broccoli can be reduced by 138 kg ha⁻¹ compared to winter fallow or non-legume cover crop.

Self-regenerating annual forage legumes, such as subterranean clover and various *Medicago* species constitute leys or pastures that are normally allowed to remain for several years before the next cropping phase. Thus, they can function as a sod phase and a safer, more profitable means of providing nitrogen for succeeding crops rather than fertilizers (Puckridge and French, 1983).

Sheaffer *et al.*, (2001) reported that annual medics and clover have the potential to diversify crop rotations, produce forage and nitrogen and affect yields of a subsequent crop while the impact of this legume nitrogen on yield of a subsequent grain crop will be most evident in organic production systems where no synthetic nitrogen fertilizers are applied and on soils with a low nitrogen status such as the loamy sand soil.

In recent study performed by (Jamea, 2004), The marketable yield as well as all growth parameters of lettuce was significantly increased by the effect of the preceding self-reseeding legumes in average 52% higher than the control.

In similar study, Al-Bitar (2005) reported that all annual legumes that were planted had significant positive effect on both subsequent crops (lettuce and maize) and that a marketable yield of lettuce increased an average of 55% over the control and yield of maize grain was an average of 15% over the control.

Furthermore, in another study done by Caporali *et al.*, (2004) aimed to evaluate the effects of the incorporation of different winter cover crops on maize yield and weed suppression, results have shown that soil nitrogen inputs after subclover or hairy vetch green manuring were generally sufficient to replace an application of 100 to 200 kg ha⁻¹ of nitrogen synthetic fertilizer, which is more than the recommended nitrogen rate for maize.

Caporali *et al.*, (2004) also reported that the amount of nitrogen contributed by the cover crops depended upon the species and the weather trend that determined vigour and duration of the cover crop growth cycle and that the non-leguminous cover crop green manure had a little effect on subsequent maize yield and grain and straw nitrogen accumulation, compared to green manuring by leguminous cover crops. Weed growth in maize was also affected by cover crop green manuring, their aboveground biomass and density being lower with the selected cover crops than with the control treatment. These results suggest that legume green manuring can allow good yield of maize while helping to make weed control successful.

1.4.7. Tested species (Zohary, 1987)

1.4.7.1. *Medicago* species

A- *Medicago polymorpha* L.

Annual plant, 10-30 cm height. Have many stems. Procumbent or ascending. Branching from base. Leaflets 0.8-2*0.7-1.5 cm. Obviate, usually retuse. Peduncles shorter or longer than petiole. Flowers 3.5-6 mm (March-April). Pod 0.2-1.2 cm thick and 0.35-1 cm in diameter at the broadest coil, discoid to cylindrical, glabrous, coils 1.5-6, loose, soft or indurated at maturity, coil surface with many radial veins. Seeds 1-2 in each coil, 2-4 mm, yellow to yellowish-brown, smooth, radicle about half as long as cotyledons.

B- *Medicago radiata* (L.) Boiss

Annual plant, 10-25 cm height. With prostrate or ascending stems, leaflets about 0.6-1*0.4-0.6 cm. peduncles shorter than to nearly as long as leaves. Flowers 5-6 mm, flowering is in the period between March and May. Pod 1.5-2 cm in diameter, membranous, with dense radial veins, coils 0.5-1.5. Seeds 4-7, up to 3 mm, ovoid yellowish brown, wrinkled.

C- *Medicago rigidula* (L.) All.

Annual plant, hairy or glandular-hairy, 10-25 cm height. Stems procumbent, branching from base, leaflets 4-8*3-6 mm. Obviate-cuneate to broadly obviate, obtuse to truncate, serrate-dentate above. Peduncles usually longer than petiole. Flowers 6-8 mm (March-May). Pod 0.5-1.2 cm thick and 0.5-1 cm in diameter, discoid, cylindrical, rarely ovoid, much indurating at maturity, hairy or glabrescent, with spiny, tuberculate or smooth margins, coils 4-7, coil surface with 12-17 strongly bent radial veins. Seeds 1-2 in each coil, 3-4 mm and yellow to yellowish brown, smooth.

1.4.7.2- *Trifolium* species**A- *Trifolium cherleri* L.**

Annual plant, 5-20 cm height. Stems few to many, ascending or procumbent, rather thick. Leaves long-petioled, leaflets 0.6-1*0.4-0.5 cm, many-flowers. Flowers 6-8 mm (March-April). Pod membranous, ovoid, with an operculum and oblique style. Seed 1, ovoid-reniform, brown shiny.

B- *Trifolium campestre* Schreb.

Annual plant, hairy or almost glabrous, 10-30 cm height. Stems ascending or prostrate, simple or branched. Leaves petiolate, leaflets 0.8-1.6*0.4-0.8 cm, ovate to oblong-elliptical, terminal leaflet long petiolulate. Peduncles as long as, longer or shorter than leaves. Heads 0.8-1.3*0.7-1 cm. Many-flowered, often globular. Flower (April) rather dense, later becoming densely imbricated. Pod longer than style, stipitate. Seeds solitary, 1 mm, ovoid-lenticular.

C- *Trifolium stellatum* L.

Annual plant, 10-20 cm height. Few stems, mostly erect or ascending. Leaves long petiolated, stipules membranous ovate, dentate, green at margin, leaflets mostly 5-8*4-8 cm, dentate at upper part. Heads 1.5-2 cm, long peduncled, many flowered. Flowers 1.5-1.8 cm (February-April), loose. Pod short-stipitate, membranous, lanceolate to pear-shaped. Seeds solitary, shiny, yellowish.

D- *Trifolium angustifolium* L.

Annual plant, 10-30 cm height. Solitary or few stems, ascending or erect, scarcely branched. Lower leaves short-petioled, upper leaves long-petiolated, leaflets 3-5*0.2-0.4 cm. Narrowly linear-lanceolate, those of upper leaves acute, those of lower leaves obtuse. Heads 2-6 cm, short peduncled, spike-like, cylindrical or conical. Flowers 1-1.3 cm (March-April). Pod ovoid, membranous. Seed 1, ovoid, light brown.

E- *Trifolium subterraneum* spp. *Brachcalycium* (cv. Antas)

Annual plant, 10-30 cm height. Few stems, seak, slightly furrowed. Leaves long-petioled, leaflets 0.8-1.2 cm, mostly obcordate with cuneate base, denticulate and mostly notched at apex. Heads about 1 cm, long-peduncled, obvoid, loose, the fruiting heads globular. Flowering (March-April), fertile flowers few or many, flowers several, developing after anthesis of the fertile ones. Pod membranous. Obvoid or obtriangular. Seeds solitary, 2.5 mm, lenticular, black.

1.5. Subsequent crops**1.5.1. Zucchini (*Cucurbita pepo* L.)**

Zucchini is one of summer squashes that are a member of Cucurbitaceae family. Cucurbitaceae is mostly prostrate or climbing herbaceous annuals comprising about 90 genera and 700 species and considered as a medium-sized plant family, primarily found in the warmer regions of the world. It is a major family for economically important species, particularly those with edible fruits. Some of these represent some of the earliest cultivated plants in both the old and new worlds. Some have medicinal and other uses (Andres, 2004).

Many varieties of the cucurbitaceae family, including the squash, have been cultivated for so long that their wild form no longer exists and their places of origin are uncertain. It is believed that some squash varieties originated in Asia and were cultivated by Native North Americans. Some others are believed to be native to Central America and Mexico.

The annual world production of squash, pumpkins and gourds reached an estimated 8.7 million tones through the mid 1990s. China ranks number one in squash production throughout the world while Italy ranks number 8. Table 4 shows the total production of squash and pumpkin and gourds in the first ten producer countries in the year 2005 (FAO, 2005b).

Table 4: Total squash and pumpkin and gourds production in 2005

Rank	Country	Production (kg*10 ³)	
1	China	5,767,700	F
2	India	3,500,000	F
3	Ukraine	1,072,000	*
4	USA	804,260	F
5	Egypt	690,000	F
6	Mexico	560,000	F
7	Cuba	520,000	F
8	Italy	505,568	
9	Iran	505,000	F
10	South Africa	378,776	

Source: FAO datastatistics (2005b) , No symbol= Official figure, *= Unofficial figure, F= FAO estimate

1.5.1.1. Morphology and floral biology

The Cucurbitaceae is characterized by commonly having five-angled stems and coiled tendrils. The leaves are alternate and usually palmately 5-lobed or divided and stipules are absent. Squash is monoecious (the male and female flowers develop on the same plant). During the main growing season, the ratio of male to female flowers is usually 3:1 or higher. The female flower is distinguished by the presence of an ovary in the base and they are born on very short stems while the male flowers born on long stems. Honey bees are the primary pollinators and 2.5-5 hives of bees should be provided for a good fruit set. Zucchini squash is green and straight. Poor pollination results in small young fruits that turn yellow shrivel and fall off. Incomplete pollination may also cause misshapen fruits that are unmarketable. Squash fruits grow about 1.9-2.5 cm per day (Molinar *et al.*, 2000).

1.5.1.2. Climatic requirements

Summer squash is a warm-season crop, since it is a short season crop, it thrives in somewhat cooler climate better than other cucurbits such as cantaloupes and watermelon. The optimal germinating temperature ranges is 21-35°C, and the maximum germinating temperature is 38°C. Germination may take long as two weeks. The optimal growth temperature range is 18-24°C (Molinar *et al.*, 2000).

1.5.1.3. Soil requirement

Squash grows on a wide variety of soil types with proper management. In all cases, however, the soil should be well drained. Sufficient amount of nitrogen is needed to produce maximum summer squash yield. Previous crop history should also be considered when selecting a site (Boyhan *et al.*, 1999). The optimal pH is 5.8-7 (Molinar *et al.*, 2000).

1.5.1.4. Irrigation

Squash roots develop rapidly, with roots in the top 45.5 cm of soil. Irrigation should be scheduled to avoid excessive moisture or water stress. Early season irrigation tend to cool the soil and slow plant growth. At least 1.854 m³ is required for the season. Generally, 103 m³ of water is applied each irrigation. Sandy soils require more frequent irrigations than clay soils. Lack of adequate moisture at harvest can result in misshapen fruits and too much moisture can aggravate root and stem rot diseases (Molinar *et al.*, 2000)

1.5.1.5. Zucchini main pests and diseases

a- Powdery mildew

Fungal disease caused by the fungi *Erysiphe cichoracearum* and *Sphaerotheca fuliginous*. It appears as a white powder on the upper leaf surface. All cucurbit species are susceptible to powdery mildew, although resistant varieties of summer squash, winter squash and pumpkin are available. As a consequence, yields are reduced because of a decrease in the size or number of fruits or a shortened harvest period. As the disease advances, the leaves turn yellow, brown and die (McGrath and Staniszewska, 1996).

b- Downy mildew

Downy mildew is caused by the fungus *Pseudoperonospora cubensis* and is favored by moist conditions. It is one of the most important leaf diseases in cucurbits. Typically, symptoms begin as small yellow areas on the upper leaf surface. As lesions expand, they may become brown with irregular margins. Affected areas may grow together, and the entire leaf may wither and die. Infected plants also develop a gray mold on the lower leaf surface. The fruit is not affected but it will be less sweet (Boyhan *et al.*, 1999).

c- Viral diseases

Viruses are the most limiting factor to squash production, particularly during summer and fall months. There are several viruses that cause diseases in squash including cucumber mosaic virus, papaya ring spot virus Type W, watermelon mosaic virus 2 and zucchini yellow mosaic virus.

Symptoms in young infected plants may exhibit prominent vein clearing, chlorotic spotting and a mosaic on leaves. Older plants may exhibit stunting with varying degrees of mottling, leaf blistering, malformation and vein extension along leaf borders depending on the strain of virus, age of infection and possibly other factors. Yellow squash varieties will exhibit varying degrees of fruit greening in a striped or mottled pattern, sometimes with raised yellow blisters. Green-fruited squash may lighten or mottle in color as well as blister. Fruit distortion can be severe across squash types. The use of resistant varieties is the only reliable control for diseases caused by viruses (Roberts and Kucharek, 2007).

d- Aphids

Soft-bodied insects that often appear in clusters and may be green, red, brown or black. They suck plant juices and transmit viral diseases. They do not cause serious direct injury to cucurbits. Aphid feeding may cause the leaves to become distorted. Honeydew may also serve as a growing medium for sooty mould, a fungus that can disfigure the fruit with black blotches. Natural enemies will help keep aphid populations in check but will be less effective in very hot weather when aphids reproduce rapidly (Boyhan *et al.*, 1999).

e- Squash bugs

Flat-backed bugs that damage the plant by sucking plant juices. Masses of orange-yellow eggs can be seen on the underside of leaves. They emit a disagreeable odor when crushed (Boyhan *et al.*, 1999).

1.5.2. Lettuce (*Lactuca sativa* L.)

Lettuce is an annual of the Asteraceae family, and native to the Mediterranean basin (Bianco, 1990). It is thought to have originated in Europe and Asia and has been cultivated for at least 2500 year. The cultivated lettuce is a popular salad and is grown on all continents throughout the world including Italy.

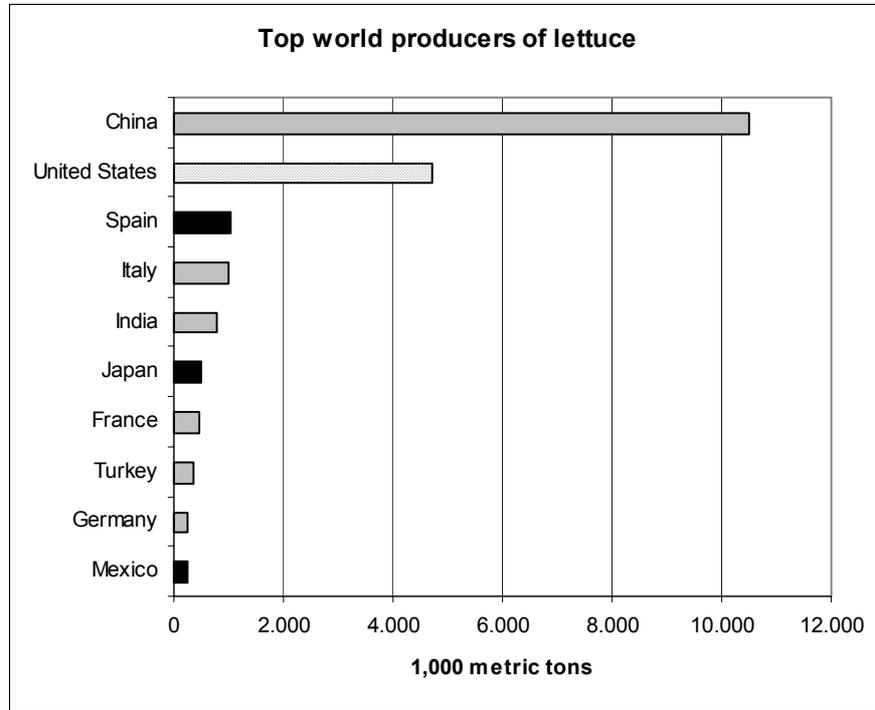
There are four main types of lettuce:

Crisphead: more commonly known as iceberg which forms a tight firm head of crisp leaves. At maturity most varieties are mid green in colour and about 1 kg in weight.

Butterhead: a heading type like crisphead but with the leaves more loosely folded on top of one another. Head sizes range from 0.5-0.7 kg for field grown cultivars

Romaine: this lettuce is upright in form and grows up to 30 cm tall. The tightly leaves are greenish white in the centre and medium green on the outside. Their flavour is sweeter than the other types of lettuce.

Loosleaf: this type does not form hearts and comes in a range of colours with various type mottling or patterns.

Figure 3: Top world producers of lettuce crop

Source: FAO data statistics (2005c)

1.5.2.1. Planting and soil conditions

Field grown lettuce is planted from February onwards. Early crops are frequently covered with fleece or polyethylene. It has a very short growing season and a shallow root system at harvest, planting to harvest takes 60 to 80 days for midsummer plantings and as long as 150 days for late-fall plantings (Bianco, 1990). It can be grown on a wide variety of soil types. Very heavy soils are best avoided as these present difficulties at both planting and at harvest. Very light soils are best avoided when growing Iceberg lettuce. Lettuce prefers a sandy-loam soil high in organic matter. Lettuce is sensitive to soil acidity; lime should be added, if necessary, to adjust the pH to 6.5-7.0. Lettuce is also sensitive to excess salts, especially at germination. If the previous crop was heavily fertilized, leaching the soil through heavy irrigation might be advisable. Field-grown lettuce, especially, requires good drainage to avoid certain fungal diseases such as bottom rot. A good soil preparation is required. Generally soil tillage is about 40 cm depth and must be realized at least 2 weeks prior to lettuce transplanting. Plant density is correlated to soil preparation and lettuce cultivar. For Iceberg and Romane seedlings can be arranged in rows spacing 35-50 cm and 20-35 within plant (about 6-14 plants m⁻²) (Bianco, 1990).

1.5.2.2. Irrigation requirements

Correct management of irrigation is an integral part of growing outdoors lettuce. When lettuce is grown blocks or modules, it is essential that rapid establishment is achieved. This invariably means that suitable quantities of water are applied to the crop soon after planting. Once the crop is established, irrigation requirement will vary according to the soil type, crop growth stage and prevailing weather conditions. The amount of 15000-2000 m³ ha⁻¹ is typically used to grow a lettuce crop in more or less dry conditions (Bianco, 1990). The majority of the water is applied in the last 30 days before harvest. Water is a valuable resource and the use of drip irrigation should be considered

1.5.2.3. Lettuce main pest, disease, weed and physiological disorder

Pest: A number of aphid species attack Lettuce. Aphids can make lettuce totally unmarketable as well as spreading virus diseases. The two most commonly occurring species are the lettuce currant aphid (*Nasonovia ribisnigri*) and the peach-potato aphid (*Myzus persicae*). The lettuce root aphid is also a serious pest in some areas. There are now several cultivars of lettuce which offer resistance to the lettuce currant aphid. These cultivars are already widely available for 'iceberg' types and some are gradually becoming available for other types of lettuce such as Romaine and Little Gem.

Slugs have become an increasingly important pest of field grown lettuces over the last few years. A succession of mild winters has ensured high survival rates and the straw burning ban has meant that those growers in a cereal rotation face dealing with increasing amounts of trash which encourages overwintering populations (Bianco, 1990 and Kuepper *et al.*, 2007).

Disease: While few diseases other than damping-off affect *young* lettuces and greens, older plants face more challenges. The limited number of fungicides and other disease control agents available to organic growers makes disease prevention crucial in organic production. The cool moist conditions typical of fall and spring production may encourage diseases in the field. *Sclerotinia* drop is caused by fungi that attack the lower leaves in contact with the soil, producing a cottony growth. Eventually the entire plant may “collapse,” with the leaves dropping down on one another in succession. Bottom rot, caused by *Rhizoctonia* species, is another potentially serious soil-borne fungal disease of cool-season lettuce. It occurs on nearly mature plants, first appearing as slightly sunken rust-colored lesions (plus or minus some amber ooze) of varying size. Affected plants will have a very short shelf life if harvested, and may decompose into a slimy black mass (Bianco, 1990 and Kuepper *et al.*, 2007).

This virus disease “Beet western yellow virus” causes interveinal yellowing and is sometimes confused with magnesium deficiency. Aphids spread the virus and as the virus are retained by the aphid for long periods widespread and long distance infection can take place. Ringspot (*Microdochium panattonianum*) is a disease that sometimes

referred to as lettuce rust. It can occasionally be serious, especially on Little Gem and Cos-type Lettuces. Plants exhibit small sunken brown spots on the leaves and midribs, which is occasionally mistaken for slug grazing. The disease is particularly bad in wet, mild seasons. Big vein is a widespread virus disease of Lettuce, which is carried by the fungus, *Ospidium brassicae*. The problem is often most evident in early and late season Lettuce, i.e. when the temperature is cooler (Bianco, 1990 and Kuepper *et al.*, 2007).

Weed: Many greens crops are poor competitors with weeds. Additionally, weeds growing within rows can inadvertently be harvested along with the crop and contaminate the product. As a result, considerable effort may be expended in cultivation and handweeding to assure a clean field (Kuepper *et al.* 2007).

A disease (actually, “**physiological disorder**”) of particular concern on lettuce is tipburn. Tipburn occurs when a sudden change in weather causes more rapid transpiration than water uptake. Symptoms include browning and rotting of the edges of internal leaves, which may not be visible from the outside of the head. This disorder is related to calcium deficiency and is aggravated by high soil fertility and high temperatures. On greenhouse lettuce it is particularly a problem during sunny early-spring days; in the field, it can be a problem if irrigation is mismanaged during hot weather. Assurance of adequate calcium and avoidance of excess nitrogen and potassium will work to minimize problems. Minimal venting (thus decreased transpiration) during the critical period is also important for greenhouse-grown lettuces. Shading may also be used to reduce transpiration, as may watering by mist or spray as long as it doesn't compromise disease control. Acid soils and high soil salt content increase risk (Bianco, 1990, and Kuepper *et al.*, 2007).

Crop rotation will help the build up of pests and disease. The length of the rotation will depend on several factors such as availability and suitability of site, water resources and management available. So many factors influence the production of field-grown lettuce that although one year lettuce crop are generally attainable, the more beneficial longer breaks are not always achievable.

1.6. Legumes-based vegetables rotation programme

Over the last two decades, significant research efforts have been devoted to sustainable farming systems for agronomic crops. Vegetable growers have become increasingly interested in sustainable production systems (Hutchinson and McGiffen, 2000; Ngouajio and McGiffen, 2003). This trend has been encouraged by several factors: few herbicides are registered for use on vegetables (Hutchinson and McGiffen, 2000); there are growing concerns about the effects of conventional production systems on the environment and health (Buys, 1993; Bond *et al.*, 1998; Clark *et al.*, 1999; Liebman and Davis, 2000); and the higher prices commanded by organic produce make organic vegetables a specialty crop that can help ensure profitability (Batte *et al.*, 1993).

More recently, the threat of methyl bromide loss coupled with the lack of immediate replacements has created demand for non-chemical alternatives. Both conventional and organic vegetable growers have integrated crop rotation, cover crops, and other weed control practices into their cropping systems (McGiffen *et al.*, 2000; Ngouajio and McGiffen, 2003). However, growers are anxious about the effects of new production practices on weed population dynamics and management. Weed control is a serious limitation to vegetable production (Clark *et al.*, 1999; Hutchinson and McGiffen, 2000) and is ranked as the top priority in organic agriculture (Davies *et al.*, 1997). Changes in the cropping system may cause significant modifications of the agroecosystem (Cuevas *et al.*, 2000; Magdoff and Van Es, 2000). Cover crop and crop management system can affect weed populations and yield in the short and long term. Short-term effects are particularly important to growers who want to convert from conventional to organic production systems.

Information on root growth is useful to design crop rotations with low N leaching losses and high overall ones (Thorup-Kristensen and Grevsen, 1999). By placing deep-rooted crops or catch crops at points in the rotation where available N is present in deeper soil layers, total losses can be reduced significantly. By placing shallow rooted crops only where little N is available in deeper soil layers, N losses after these crops can be reduced. Growing catch crops can create a situation where most of the available N is present in upper soil layers with little in deeper soil layers (Thorup-Kristensen and Nielsen, 1998), and catch crops are thus of special value when grown before shallow rooted main crops. Lettuce takes part of the second group and is advised to place it in among the least in crop rotation.

In organic vegetable production, optimising N use efficiency is especially important, both in order to protect the environment and to secure N supply for the crops. Catch crops and green manures can be used to reduce N losses and increase N supply for the crops, but they will affect the depth distribution of the available N as well as the total amount of available N in the soil (Thorup-Kristensen and Bertelsen, 1996).

Thorup-Kristensen (2001) carried out a study aiming at a rotation programme of legume-lettuce, and demonstrated that legumes left on average of 70 kg N ha⁻¹ in the soil at harvest. Moreover, organic mulches can help moderate soil temperature and the microenvironment to produce quality lettuce in less than ideal weather conditions.

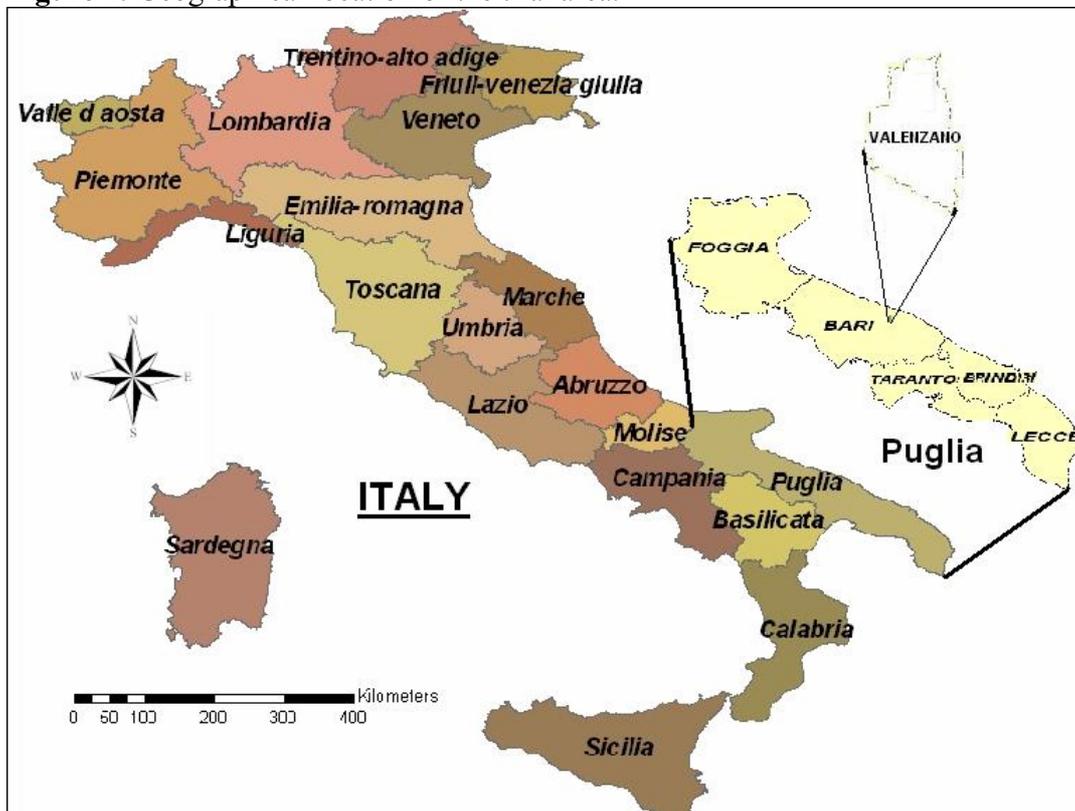
Chapter 2: Materials and Methods

2.1. Description of trial site

2.1.1. Location

The experiment was carried out at the experimental farm and laboratories of Mediterranean Agronomic of Bari (IAMB) located in Apulia region, eastern part of South Italy (Figure 4), 72 m above the sea level, latitude 41°03'16" North and longitude 16°52'45" East Greenwich (Steduto *et al.*, 2004).

Figure 4: Geographical location of the trial area.



2.1.2. Climatic conditions of the region

Apulia region is characterized by a Mediterranean climate with humid mild winter and hot dry summer. The yearly precipitations vary from 400 to 500 mm and are mainly concentrated between October and April. The annual average temperature ranges from 15 to 16 °C, with a maximum of 35 °C recorded in July and a minimum one of 0 °C recorded in January (Steduto *et al.*, 2004).

Climatic data during the study were recorded at the meteorological station located in the experimental farm of IAMB in order to analyse the climatic effect on plant behaviour during the experiment (Figure 5 & Figure 6).

Figure 5: Climatic conditions during the first legumes cropping cycle (2005-2006)

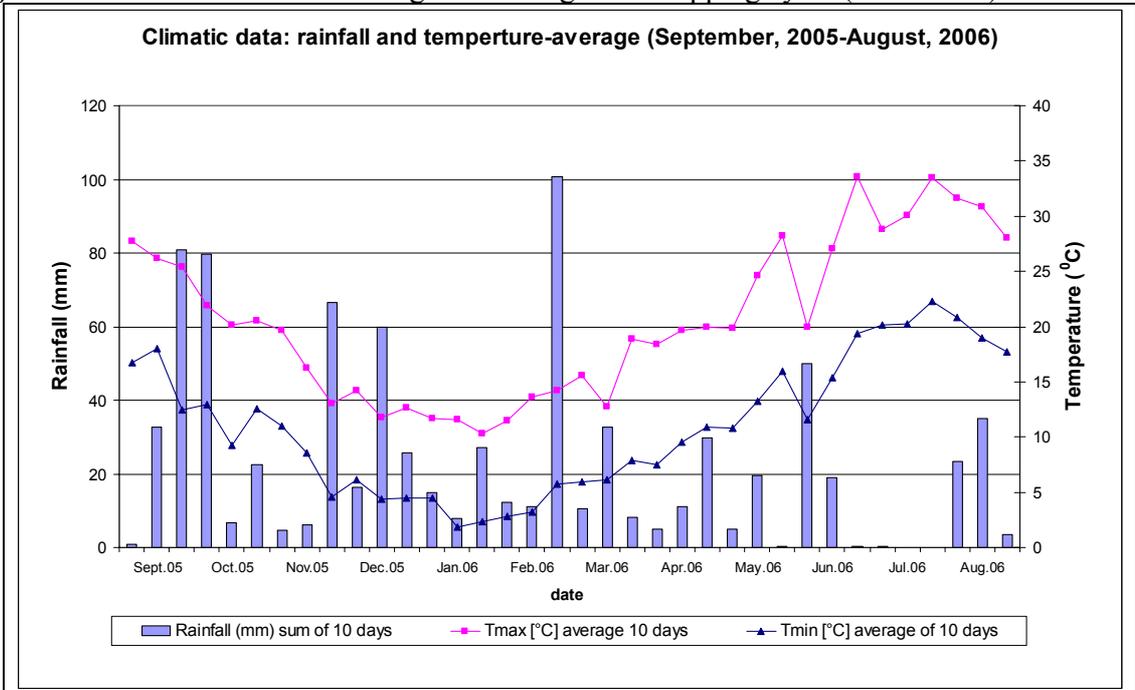
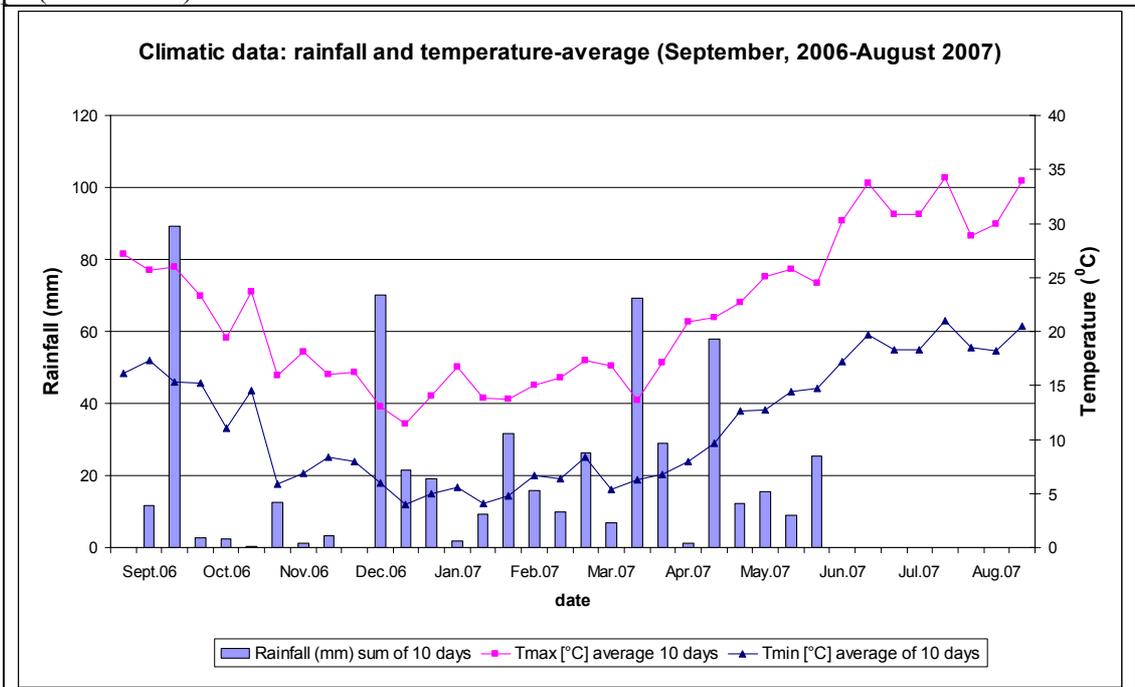


Figure 6: Climatic conditions during the legumes second cropping cycle and subsequent crops (2006-2007)



2.1.3. Soil characteristics of the experimental field

In order to identify the general site characteristics, soil analysis for the experimental site was performed before sowing (October 2005) and also during the self-establishment period of legumes (November 2006).

Composite soil samples were taken at a depth of 0-20 cm over the experimental blocks using a soil auger by making a random pattern over each plot. Five samples were taken, in each plot, mixed together in order to get one representative sample.

Samples were analysed for total nitrogen, phosphorous, potassium, soil pH, electrical conductivity (ECe), soil texture, organic matter and total carbonate in order to determine general site characteristics (table 5).

Table 5: General soil chemical and physical characteristics

	2005-2006		2006-2007	
Textural Class (USDA)	Silty loam			
		0-20 cm		0-20 CM
Stones and gravel	g kg ⁻¹	204		
pH (CaCl ₂)		7,6		7.7
pH (H ₂ O 1:2.5)		8.1		8.3
ECe dS/m 25°C		0.41		0.49
Total Carbonate	g kg ⁻¹	305	g kg ⁻¹	300
Organic Carbon	g kg ⁻¹	12.8	g kg ⁻¹	13.4
Organic Matter	g kg ⁻¹	22.0	g kg ⁻¹	23.1
Available Phosphorus	mg kg ⁻¹	28	mg kg ⁻¹	37
Available P ₂ O ₅	mg kg ⁻¹	64.12	mg kg ⁻¹	85
Exchangeable potassium	mg kg ⁻¹	78.2	mg kg ⁻¹	514
Total Nitrogen	g kg ⁻¹	0.9	g kg ⁻¹	1.0
C/N		14.2		13.4

2.2. Description of the study

The present study is of a three-year investigation programme (Table 6) that aims at screening seven Mediterranean native legume species in order to integrate the most suitable ones into organic cropping systems.

Table 6: Description of the rotation programme (2005-2007)

First cropping cycle (2005-2006)	Second year cropping cycle (2006-2007)		
		Subsequent crops	
Legumes screening	Self-establishment (Legumes screening)	Zucchini	Lettuce
Nov.05-June 06	October 06 – April 07	April07-July07	July07-agust07

2.3. Plant materials

The tested legumes species (Table 7) native to the Mediterranean region were selected from the germplasm collection of the International Center for Agricultural Research in the Dry Areas (ICARDA). They were collected from the mountain of eastern part of the Mediterranean, characterized by a resistance to cold and tolerance to low rainfall, reproduced in Tel Hadya-Aleppo valley and still under ICARDA's forage breeding programme. The first year experiment (2005-2006) aimed at the screening of those native species at sowing rate of 28 kg ha⁻¹. They were established in October 2005 and grown during the period between October 2005 and June 2006. In the summer, legumes were left on the field after seed production. In autumn (October 2006), legume self-established after the first rain and grew until April 2007. Legumes were then incorporated as a green manure for the purpose of investigating their effect on subsequent crops.

Table 7: Tested species (Treatments)

Tested species			
Genus	Specie	Common name	Abbreviation
<i>Medicago</i>	<i>polymorpha</i> (L.)	Burr medic	<i>M.p.</i>
<i>Medicago</i>	<i>radiata</i> (L.) Boiss	Ray-podded medic	<i>M.ra.</i>
<i>Medicago</i>	<i>rigidula</i> (L.) All.	Tifton burclover	<i>M.ri.</i>
<i>Trifolium</i>	<i>cherleri</i> (L.)	Cupped clover	<i>T.ch.</i>
<i>Trifolium</i>	<i>campestre</i> Schreb	Hop clover-field	<i>T.ca.</i>
<i>Trifolium</i>	<i>stellatum</i> (L.)	Starry clover	<i>T.st.</i>
<i>Trifolium</i>	<i>angustifolium</i> (L.)	Narrow leaf	<i>T.a.</i>
Controls			
<i>Trifolium</i>	<i>subterraneum</i> cv.ANTAS control	Sub clover	<i>Crl 1 (T.su.)</i>
	<i>Weed complex</i>		<i>Crl 2</i>
	<i>Bare soil</i>		<i>Crl 3</i>
<i>Hordeum</i>	<i>vulgare</i>	Barley	<i>Crl 4</i>

2.4. Experimental design

All treatments (legumes and controls) were arranged in a randomized complete block design with four replicates, each block containing 11 plots of 9 m² (3 x 3) each.

Four controls were introduced in three plots:

- Control 1: *Trifolium subterraneum* cv. ANTAS was introduced as standard control (Std-Ctrl) for its ability to grow well in both acidic and calcareous soils.
- Control 2: Natural weed complex where no legumes were grown and weeds were allowed to grow during whole legumes growth cycle. Once zucchini and lettuce were transplanted, manual weeding was done for all treatments including this control.
- Control 3 : bare soil where no legumes were grown and soil was kept clean from weeds during whole legumes growth cycle. The aim of its introduction was to study zucchini crop parameters following tested legumes in comparison with zucchini following this control.

The aim of introducing control 2 and 3 was to study subsequent crop parameters (zucchini and lettuce) following tested legumes in comparison with control without legumes preceding.

- Control 4: planted with barley (*Hordeum vulgare*) which considered as a reference crop (non N-fixing crop) for the ¹⁵N isotope dilution method analysis in order to quantify the amount of nitrogen fixed by legumes.

Barley was sown 2 times on the 3rd of November 2005 during the sowing of legumes and on the 7th of November 2006 during the their self-establishment period at rate of 180 kg ha⁻¹.

2.5. First cropping cycle: Legumes screening

2.5.1. Viability and germination tests

These two tests were conducted in accordance with the "International Seed Testing Analysis" (ISTA).

2.5.1.1. Viability test using Tetrazolium

The main objective of this test is to know the percentage of viable seeds (Alive embryos). 100 legume seeds from each species were soaked overnight in water and then boiled in water for 2 min (boiled control). Grains were cut in half and one half was discarded, then half of the seed was placed in Petri dish containing 0.1% Tetrazolium (cut side was placed down to incubate for about 30 min). At the end the embryo was examined for Tetrazolium staining.

2.5.1.2. Germination test

The direct and real goal of the germination test is to predict potential seed germination on the field in order to know if some species need to be treated before sowing to help them to germinate. Four treatments were used with three replicates (Table 8).

Table 8: Treatments used for germination test

T1	Pre-cooling -10°C 2 days
T2	mechanical disruption of seed coat by sandpaper
T3	immersion in hot 75 °C water for 5 min
T4 Control	Untreated: germinated in distilled water

50 seeds/species were put in Petri dishes to imbibe on paper bed and kept in dark at 20 °C to germinate. Data were collected after 4, 10 and 15 days.

2.5.2. Legumes establishment

False sowing (29/9/05) was done to manage weeds before sowing legumes.

Trial land was cleared and the soil was prepared to a fine tilth (Mechanical soil bed preparation on 2/11/05), before demarcating plots and sowing.

Following to the results of germination test in which *Medicago radiata* and *Trifolium campestre* species had the lowest percentages of germinated seeds, they were scarified to enhance their germination, by rubbing them between two sheets of sand paper until one or two seeds break.

Legumes and barley were sown on 3/11/05. For legume, sowing was done by a broadcast way with a rate of 28 kg ha⁻¹; the homogenous distribution of seed was aided by mixing them with sand before sowing. For barley, sowing was done manually (180 kg ha⁻¹) per line (11 lines subplot⁻¹, 7.4 g of seeds per line and 10 cm of distance between lines).

No fertilisers and no inoculants were used with the aim to identify species which could survive even under harsh conditions with some mismanagement.

At the beginning, plots were kept free of weeds to avoid competition, especially for slow-growing species. The paths or buffer between plots were hand weeded during all season.

2.5.3. Agronomic performance of legumes

2.5.3.1. Plant growth evaluation

The following parameters were assessed:

1 - Plant height was measured each two weeks by taking the height of three plants using a quadrat of 1 m² thrown randomly in each plot.

2 - Percentage of herbaceous ground cover was done visually every two weeks from the same quadrat used for plant height evaluation.

3 - Flowering dates: first flowers, 50 and 100 % flowering stages were monitored visually each day after the appearance of first flower. Number of days was calculated starting from planting day, to distinguish between early and late flowering species.

4 - Full maturity date: was monitored visually each day after full-flowering stage. Number of days after sowing was calculated.

5 - Dry biomass: at the end of legumes cycle, representative samples were taken from each plot and dried in the oven at 65C° for dry matter content.

6 - Seed yield quantification: After full-maturity, plants were cut from top soil using a triangle of 0.5 x 3 m from the internal part of each plot inside each block. Samples were dried and seeds were extracted from pods manually. Seeds of each plot were weighed.

2.5.3.2. Biological nitrogen quantification (BNF)

The amount of fixed nitrogen symbiotically was done using ¹⁵N isotope dilution method: A quadrat of 2.25 m² was identified before flowering stage, in each plot. The identification was based on the highest concentration of plants in each plot. Two grams of ammonium sulphate (¹⁵NH₄)₂SO₄ diluted in 10 liters of water were sprayed, inside quadrates (3/4/06), then, plants were washed by 10 liters of water to allow the product to penetrate into the soil.

At full vegetative activity of all species (including barley) and after full-flowering stage (9/5/06), 10 plants were taken from each identified quadrat to determine fresh and dry weight, total nitrogen and ¹⁵N quantification after mixing all parts of the plant in order to have a representative sample.

Total nitrogen content was identified by distillation method using 0.5 g of dry plant material mixed with 15 ml of acid solution (H_2SO_4 + Acid salicil 2,5 %), selenium and zinc. The mixture was kept for 6 h to have a total digestion of plant material, and then percentage of total nitrogen content was measured.

^{15}N quantification was done using the mass spectrophotometer method. Drying and grinding were done for all plant parts; 1mg of each sample was inserted into small tin capsule. Once the appropriate amount of sample was inserted, the capsule was crushed into a small squares or balls (Figure 7). At the end, capsules were placed in the mass spectrophotometer that gives the readings of atom ^{15}N percentage (AT ^{15}N %) in the plant (Figure 8).



Figure 7: mini-capsules preparation



Figure 8 : Insertion in spectrophotometer

Fixed nitrogen quantification was determined by two steps (Danso *et al.*, 1985; Unkovich and Pate, 2001):

1- Calculating the % isotopic excess by the following formula:

$$\% \text{ isotopic excess} = AT \text{ } ^{15}N\% - 0.3663*$$

*0.3663 is a constant of ^{15}N atoms ratio in the air and used for legumes to get the % of isotopic excess.

2- Calculating the percentage of the nitrogen derived from the atmosphere (%Ndfa) which is the percentage of total fixed nitrogen found in the aerial part of the plant:

$$\%Ndfa = [1 - (\% \text{ isotopic excess}_{\text{(species)}} / \% \text{ isotopic excess}_{\text{(Barley)}})] \times 100$$

At the end, the fixed nitrogen ($kg \text{ ha}^{-1}$) of each species was calculated according to the following formula:

$$N \text{ fixed } (kg \text{ ha}^{-1}) = N \text{ uptaken} * (kg \text{ ha}^{-1}) \times \%Ndfa / 100$$

* N uptaken = dry matter ($kg \text{ ha}^{-1}$) \times plant total nitrogen content (%).

2.5.4. Weed occurrence and disease incidence

Manual weeding was done during the last week of January and the first week of March. During the first weeding a quadrat of 1 m² was thrown randomly in each plot and weed species were identified and counted to calculate the weed biodiversity of the field and the weed community structure of each legume species (Bàrberi *et al.*, 1998).

Weed diversity: The Shannon index (also called the Shannon–Weaver index or the Shannon Wiener index) H' is one of several diversity indices used to measure biodiversity. The advantage of this index is that it takes into account the number of species and the evenness of the species (Equitability index) E . The index is increased either by having more unique species, or by having a greater species evenness.

Weed community structure: was measured using the Shannon diversity index (H') of each legume species.

$$H' = -\sum_{i=1}^S p_i \ln p_i$$

- p_i : The relative abundance of each species, calculated as the proportion of individuals of a given species to the total number of individuals in the community: n_i/N
 - n_i : The number of individuals in each species; the abundance of each species.
 - N : The total number of all individuals.
 - S : The number of species. Also called species richness.

$$E = H' / \ln(S)$$

Disease incidence was monitored visually each two weeks.

2.5.5. Soil physical and chemical properties

2.5.5.1. Soil sampling

Soil sampling was done before the legumes establishment after soil ploughing (November 2005) and before legumes self-establishment (October 2006). Composite soil samples were taken at a depth of 0-20 cm over the experimental blocks using a soil auger, by making a random zigzag pattern over the entire block. Five samples were taken, in each plot, mixed together in order to get one representative sample.

Samples were put in plastic bags, weighed and transferred to greenhouse where they were left for two days in order to dry up and then weighed. Soil was then, sieved using a normal sifter ($\Phi < 2$ mm) to separate “Skeletron” and fine soil.

Samples were analysed for nitrogen, phosphorus, potassium soil pH, electrical conductivity (ECe), texture and organic matter content and total carbonate in order to determine general site characteristics (Table 5).

2.5.5.2. Soil analysis procedure

- **pH** was measured in a 1: 2.5 soil suspension using a pH meter as described by Rhoades (1982).
- Total soluble salt (**ECe**) was determined in the soil saturation extract using electrical conductivity meter.
- Mechanical analysis for soil **texture** was performed by the pipette method using the hexametaphosphate as dispersing agent according to the description reported by Chapman and Pratt (1961).
- **Organic matter** content was analysed according to the Walkley and Black method (Jakson, 1967).
- **Exchangeable potassium** was determined by shaking 2.5 g of soil with 50 ml of BaCl₂ solution for an hour, then, the extract was filtered using Whatman filter paper, and the potassium was measured in the filtrate using the flame photometer according to Black *et al* (1982).
- **Available phosphorus** was determined by shaking 5 g of soil with 100 ml of NaHCO₃ 0.5 N for 30 minutes, then, the extract was filtered using Whatman filter paper. Phosphorus was determined in the filtered extract calorimetrically by spectrophotometer using the ascorbic acid method described by Jackson (1958).
- **Total carbonate** in the soil was determined using the Dietrich-Fruling gasometer calcimeter by measuring the volume of CO₂ developed after adding a plenty amount of diluted 1:1 HCL acid to about 1 g soil.
- **Nitrogen analysis**: An automatic steam-distilling unit was used for the analysis of nitrogen in soil extracts; the Kjeldahl’s method is still the most suitable method for determining nitrogen due to precision and reproducibility.

2.6. Second cropping cycle: Legumes self-establishment

At the end of their first cropping cycle, legumes after seeds production were left on the field to ensure their self-establishment and the regeneration during the next season.

This work will focus on screening the regenerated legumes in their second cropping cycle to assess their self-establishment capacity, as well as their impact on zucchini and lettuce as subsequent crop within the rotation programme.

Legumes started emergence late in October 2006, grown until the third of April 2007 then, they were cut and incorporated in the soil (figure 9). Two subsequent crops were introduced after legumes. Zucchini was established about two weeks after legumes incorporation and lettuce was transplanted ten days after the end of Zucchini growth cycle (10/07/2007).

Two species belonging to the genus *Medicago* (*Medicago rigidula* and *Medicago radiata*) did not regenerate at all. They were excluded from the study since the absence of germination was enough to indicate that they will not perform well for the purpose of green manuring when integrated into a rotation programme.

2.6.1. Legume plants analysis

In order to study self-establishment capacity of legumes, the following parameters were measured:

2.6.1.1. Number of plants

Number of germinated plants was counted every two weeks in order to identify the self-establishment capacity of the tested legumes, using a quadrat of 0.25 m² fixed on each plot.

2.6.1.2. Percentage of herbaceous ground cover

Herbaceous ground cover was estimated visually every two weeks from the same quadrat used for calculating number of germinated plants.

2.6.1.3. Plant height

Legumes plants height was measured each two weeks by taking the average height of three plants also from the same quadrat used for the two previous observations.

2.6.1.4. Dry biomass

Before legumes incorporation, representative samples were taken from each plot and dried in the oven at 65°C for dry matter content.

2.6.1.5. Nitrogen fixed quantification (idem to the first cropping cycle)

The amount of fixed nitrogen symbiotically was done using ^{15}N isotope dilution method. A quadrat of 0.25 m^2 was identified in the beginning of January in all plots. Two grams of ammonium sulphate ($^{15}\text{NH}_4$) $_2\text{SO}_4$ were diluted in 10 liters of water and sprayed inside the quadrates, then, plants were washed by 10 liter of water to allow the product to penetrate into the soil. Just before green manuring (28.03.2007). Plants biomass production was estimated in the quadrat sampling area in each plot, samples were dried at 65°C until constant weight and biomass was expressed on a dry matter basis Samples then were analysed for total nitrogen and ^{15}N quantification after mixing all parts of the plant in order to have a representative sample.

To calculate plants total nitrogen, suitable weights of the dried samples were melded, digested using concentrated H_2SO_4 and H_2O_2 in the presence of a mix of copper sulphate and potassium sulphate as a catalyst. The digestion process was done using a digestion unit (BUCHI Digestion unit K-435 and BUCHI scrubber B-414).

^{15}N quantification (idem to the first cropping cycle)

2.7. Subsequent crops

The choice of zucchini and lettuce as subsequent crops was based on two raisons: (i) they are one of the most spread crops in Apulia region (ii) In a rotation programme, the two the families of Cucurbitacea and Astraceae are advisable in crops succession.

2.7.1. Zucchini crop

Zucchini is relatively heavy nitrogen feeder too so it can reflect reliable results concerning the uptake and accumulation of nitrogen. Furthermore it is a good choice for the grower who is considering organic vegetable production as it may be included early in rotations or in a conversion plan (Neeson, 2003).



Figure 9: Legumes incorporation in the soil

Zucchini transplants of the variety President F1 were purchased from an organic nursery. Transplanting was done on (20.04.2007). 100 cm were left between rows and 90 cm between plants within rows. The water supply was ensured by drip irrigation system.

2.7.1.1. Cultural practices

Hand weeding was done throughout the whole growth cycle for all treatments to avoid weed competition. Sulfur based fungicide was applied (24.05.2007) in one foliar application as the first sign of powdery mildew started to appear on the lower leaves (Figure 10).



Figure 10: Powdery mildew attack symptoms

One treatment against aphids was also done (28.05.2007) using natural pyrethrum based insecticide as zucchini leaves and flowers were infected by aphids (Figure 11).



Figure 11: Aphids attack symptoms on zucchini plants

2.7.1.2. Zucchini crop analysis

The following parameters were assessed regarding zucchini crop:

a- Number of leaves

In order to evaluate the growth and development of plants, number of leaves was counted for five plants fixed in each plot starting from ten days after transplanting zucchini. This observation was done each week until the level of stable leaves number.

b- Number of female flowers

Counting female flowers is crucial because it will determine the total final yield of zucchini. Counting was done twice a week starting from 21 days after transplanting for five plants fixed in each plot.

c- Soil cover

The calculation of soil cover percentage is an estimation of the crop vegetation that covers the soil surface. This percentage was determined using a frame of 1 m² divided into 10 equal parts, placed on plants, each part represents 10% so, the total soil cover is considered as 100%. This observation was done one time during the middle of the crop cycle for five plants chosen randomly in each plot.

d- Plants dry biomass

At the end of zucchini cycle, four plants were taken from each plot and dried in the oven at 65°C for dry matter content.

f- Number of fruits and marketable yield

Harvesting was done two to three times a week. Twenty one harvests were done starting from 21.05.2007 till 4.07.2007. Each harvest was recorded, as well as yield of each plot, the number of fruits and the production per plant.

e- Plants total nitrogen content

i- Total nitrogen content of fruits

To calculate fruits nitrogen content, five fruits were taken randomly from each plot, dried at 65 °C, grinded and mixed very well. Suitable weight of the dried samples was digested using concentrated H₂SO₄ and H₂O₂ in the presence of a mix of copper sulphate and potassium sulphate as a catalyst. The digestion process took place using a digestion unit (BUCHI Digestion unit k-435 and BUCHI scrubber B-414).

ii-Total nitrogen content in aerial plants parts (leaves and stems)

Four plants were taken off from each plot and dried at 65 °C then, grinded and mixed very well, suitable weight of the prepared samples were digested using concentrated H₂SO₄ and H₂O₂ in the presence of a mix of copper sulphate and potassium sulphate as a catalyst. The digestion process took place using a digestion unit (BUCHI Digestion unit k-435 and BUCHI scrubber B-414).

2.7.2. Lettuce crops

2.7.2.1. Lettuce transplanting

The lettuce seedlings were purchased from an organic nursery. The variety of lettuce was “Bionda degli ortolani-Rubia”, which demonstrated to be adapted not only to the climatic and soil conditions of Apulia region, but also to the period of cropping (summer).

At end of the zucchini cycle (July, 2007). Soil was prepared and tilled at depth of 40 cm. The days after the transplanting of lettuce (at three real leaves stage) was ensured in the same plots of annual self- reseeding legume that were considered as treatments, including controls (Bare soil and weeds complex). The plant density was about 10 plants m⁻² (20 cm spacing between plants and 50 cm between rows). The water supply was ensured by a drip irrigation system. Much care was devoted to water supply in order to avoid any excess causing N leaching. The hand weeding was performed during the whole lettuce cycle.

2.7.2.2. Lettuce crop analysis

The following parameters were assessed regarding lettuce crop:

a- Plant diameter

In order to evaluate the growth and development of plants, diameter of plant was measured for five plants fixed in each plot starting from ten days after transplanting lettuce. This observation was done every week until the marketable yield of lettuce.

b- Number leaves

Counting leaves number is crucial because it will determine the total final yield of lettuce. Counting was done every week starting 10 days after transplanting for five plants fixed in each plot.

c- Plant height

The measurement of plant height was also done to monitor lettuce growth level.

The height of plant was measured for five plants fixed in each plot starting from ten days after transplanting lettuce. This observation was done every week until the marketable yield of lettuce.

d- Leaf area

Leaf Area (LA) was determined directly at the end of lettuce cycle. Five plants from each plot were used to measure LA using LICOR-3100 area meter. Each plant leaf was stripped and fed through the entrance of the machine, which were linked to a kind of crude image scanner.

e- Plants dry matter & Total Nitrogen content

At the end of lettuce cycle five plants were taken off from each plot and dried at 65 °C then, grinded and mixed very well for dry matter measurement.

For total Nitrogen content, suitable weight of the prepared samples were digested using concentrated H₂SO₄ and H₂O₂ in the presence of a mix of copper sulphate and potassium sulphate as a catalyst. The digestion process took place using a digestion unit (BUCHI Digestion unit k-435 and BUCHI scrubber B-414).

f- Marketable yield

At the end of lettuce growth cycle, five plant per plot were sampled to estimated the marketable yield of lettuce

2.8. Soil fertility assessment

2.8.1. Soil sampling

Four samplings were done in order to see the effect of green manuring on soil available nitrogen evolution. At all sampling dates, samples were taken using the same procedure used in the first analysis done in November. The first sampling was done just before green manuring (**28.03.2007**), the second one two weeks following green manuring (**18.04.2007**), the third one at the middle of zucchini growth cycle (**28.05.2007**) and the last one at the end of the growth cycle (**28.06.2007**).

2.8.2. Mineral nitrogen analysis

Mineral nitrogen was measured by shaking 10 g of fresh soil sample with 100 ml of KCl (1 M) for an hour. After filtration, 50 ml of the extract was inserted in distillation tube in the presence of 0.2 g of reactive (Devarda's alloy powder), then, the distillation tube was placed in its position in the steam distillation unit as well as the Erlenmeyer flask containing 10 ml of 0.01 N H₂SO₄ solution on its platform, 20 ml of sodium hydroxide (NaOH 40%) were added by the automatic dispensing device then left for 15 minutes in order to ensure the reduction of nitrate and nitrite to ammonia. The time the reduction is

completed, distillation process started and continued for 6 minutes till about 300 ml of the condense is collected, then titration was done with standard NaOH (0.01 N).

2.9. Statistical analysis

Experimental data was subjected to analysis of variance (ANOVA). Contrasts (at 0.01 level of probability) were also performed using SAS to compare effects of different groups. Correlations were done between all parameters then linear or quadratic regressions were done for the most important correlated parameters.

The early and late species were identified at 100 % flowering stage for the choice of contrasts, as it's an important parameter for maximum nitrogen fixation.

- Early *Trifolium* (T.E.) are: *angustifolium* (a.) and *stellatum* (st.).
- Late *Trifolium* (T.L.) are: *cherleri* (ch.) and *campestre* (ca.).
- Too late *Trifolium* is: *subterraneum* ANTAS (su.).
- Early *Medicago* (M.E.) is: *rigidula* (ri.).
- Late *Medicago* (M.L.) are: *radiata* (ra.) and *polymorpha* (p.).

The contrasts (C) used in this experiment are the following:

- C1: *Trifolium* (T.) vs. *Medicago* (M.)
- C2: *T. subterraneum* ANTAS vs. other T.
- C3: T. early vs. T. late
- C4: T. early vs. T. early
- C5: T. late vs. T. late
- C6: M. early vs. M. late
- C7: M. late vs. M. late
- C8: N-pool soil vs. all
- C9: Barley vs. all (“all” refer to tested species)

Mean Treatment effects on subsequent crops and soil N were compared by a Duncan test at the 0.05 level probability (SAS V8 software).

Chapter 3: Results and discussions

3.1. Self-reseeding legumes establishment

3.1.1. Climatic conditions during the first growth cycle

Minimum and maximum air temperatures and precipitations were measured daily by the agro-meteorological station located near the experimental field.

Climatic data (Figure 5) showed that after legumes sowing there was a gradual decrease of both maximum and minimum temperatures till mid February, and then they increased gradually until the end of May with a slight decrease at the end of the growth cycle.

This gradual decrease of temperature in the first four months resulted, on one hand, in an increase of the cropping period and, on the other, in a slow growth rate of the plants; with the increase of temperatures there was an improvement in plant growth (Figure 13).

Temperatures fluctuated generally between a minimum of -3.6 °C during late January and a maximum of 35.6 °C in late May.

Legume growth cycle (03/11/05 till 10/6/06) was characterized by high precipitations (475 mm) (Figure 5) compared to the average of the last 10 years (about 360 mm).

3.1.2. Viability and germination test

3.1.2.1. Viability test using Tetrazolium

Viability test showed low percentage of viable seeds in both *M. ra.* (6 %) and *T. ca.* (13 %) (Figure 13); this could be probably explained whether by the low percentage of viable seed embryos, whether by the incapacity of penetration of Tetrazolium because of the seed coat imposed dormancy. Such hypothesis could be probably supported by the germination test .

As for the other species, *T. st.* had 89 % of viable seeds, the highest among *Trifolium* species, while for *Medics M. p.* was the highest with 77 % (Figure 13).

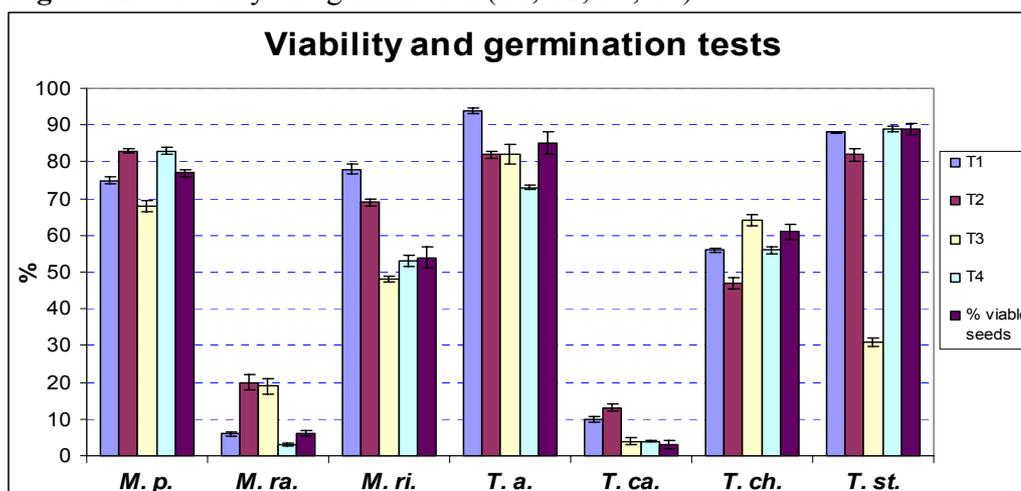
3.1.2.2. Germination test

Germination test (Figure 12) showed also low percentage of seed germination for both *M. ra.* and *T. ca.* for the four treatments used, however, these values were higher than the percentage of viable seeds. This fact could explain that the seed embryos of both species were alive but the Tetrazolium didn't penetrate the seeds because of the dormancy imposed on the embryos by the seed coat and other enclosing tissues (Taiz and Zeiger, 2002).

Figure 12 shows also that the second treatment (T2) of the germination test gave the highest percentage, even though low, of germinated seeds for *T. ca.* (14 %) and *M. ra.* (20 %) among the four used treatments. This result induced us to scarify these seeds before sowing to enhance germination on the field.

No treatments were applied for the other species as they showed more than 50 % of germinated seeds for the control treatment (T4: Untreated, germinated in distilled water).

Figure 12: Viability and germination (T1, T2, T3, T4) tests

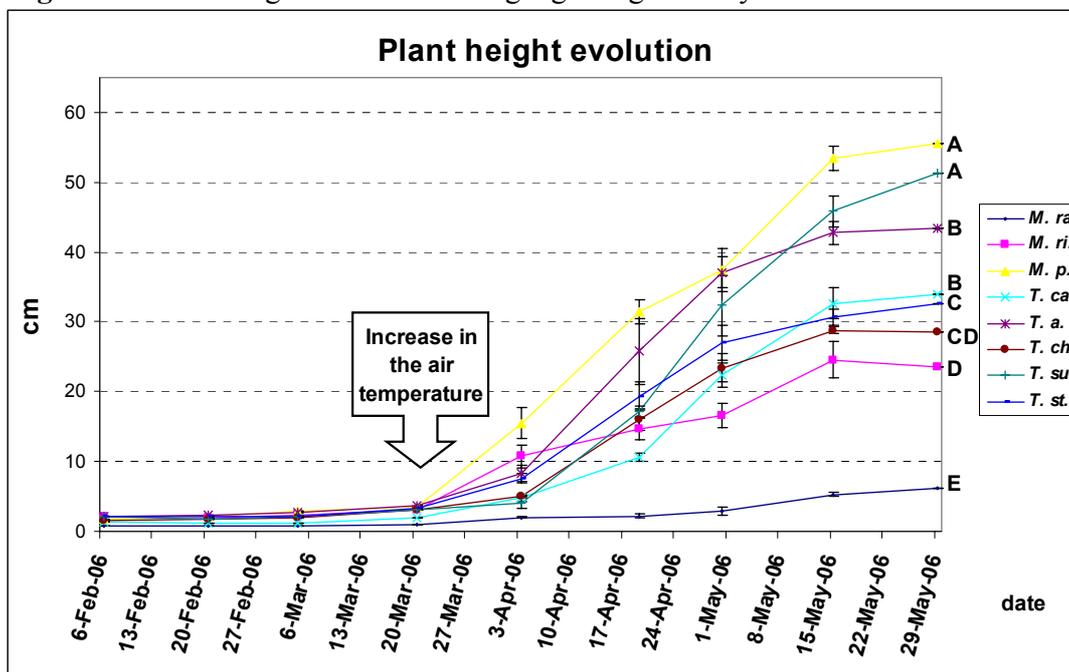


T1: Pre-cooling -10°C 2 days, T2: Mechanical disruption of seed coat by sandpaper, T3: Immersion in hot water (75 °C) for 5 min, T4 (Control). Bars correspond to the standard error.

3.1.3. Agronomic performance and biological nitrogen fixation

3.1.3.1. Plant height

The increase in plant height of legume species was very slow in the first four months, where temperatures (Figure 13) were always below the optimum needed for successful legumes growth (optimum range 20°C -25°C). These low temperatures induced also an increase in the cropping period (Figure 13). In mid march, due to the rising of temperatures, there was an important increase in plant height during the following two months to reach stability at maturity stage.

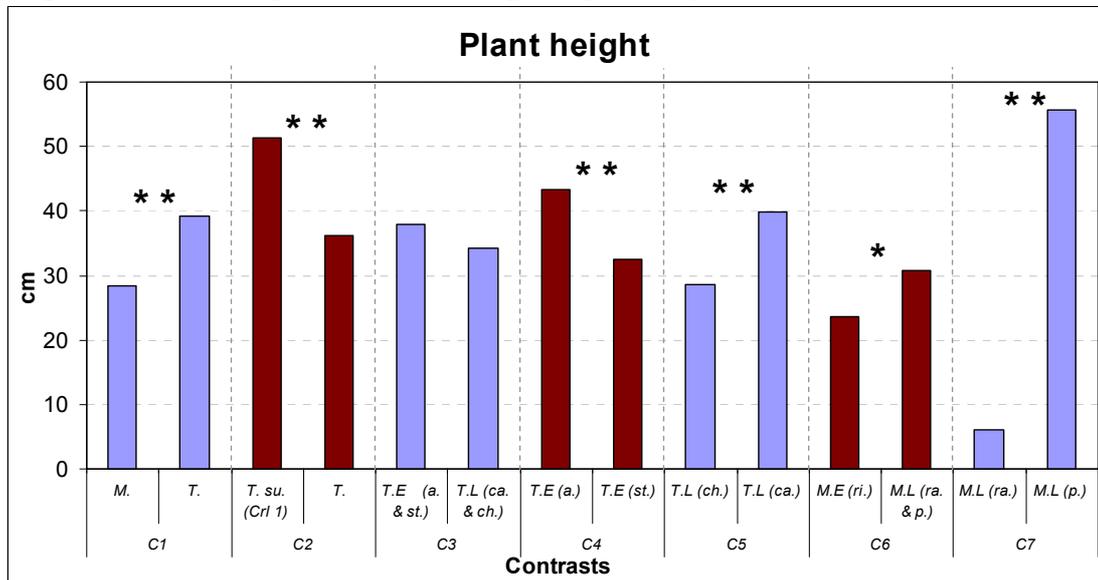
Figure 13: Plant height evolution during legume growth cycle

Bars correspond to the standard error and the values that don't have the same letters are different at 1 % (Duncan test).

Highly significant differences were obtained between the different tested legumes at the end of the growth cycle, classifying them in five different groups (Figure 13).

Positive high correlation of 82 % was observed between plant height and air temperature and 68 % with rainfall.

Regarding contrasts, statistical analysis done at the end of the growth cycle (Figure 14) showed highly significant differences between *Trifolium* and *Medicago* species, between *T. su* and the other *T.* species, between the two late *T.* (*a.* and *s.*), between the two early *T.* (*ch.* and *ca.*) and between the two late *Medicago* (*ra.* and *p.*). Significant difference was observed only between early and late *Medicago*.

Figure 14: Plant height at the end of legume growth cycle

*: Treatments are different at 5 % and **: Treatments different at 1 %.

Maximum plant height was reached by *M. p.* with 56 cm (maximum value was 30 cm as reported by Zohary, 1987 and 50 cm reported by Lonchamp, (2000) than *T. su.* with 51 cm (maximum value was 30 cm as reported by Zohary, (1987) and 25 cm reported by Lonchamp (2000) while the shortest was *M. ra.* with 6 cm (maximum value was 25 cm as reported by Zohary, (1987).

3.1.3.2. Herbaceous ground cover

Herbaceous ground cover is an important parameter to evaluate species. Plant ground cover could assure weed competition, soil protection from erosion and water loss, optimization of nutrient cycle, increase of organic matter and control of pests and insects (Campiglia, 1999).

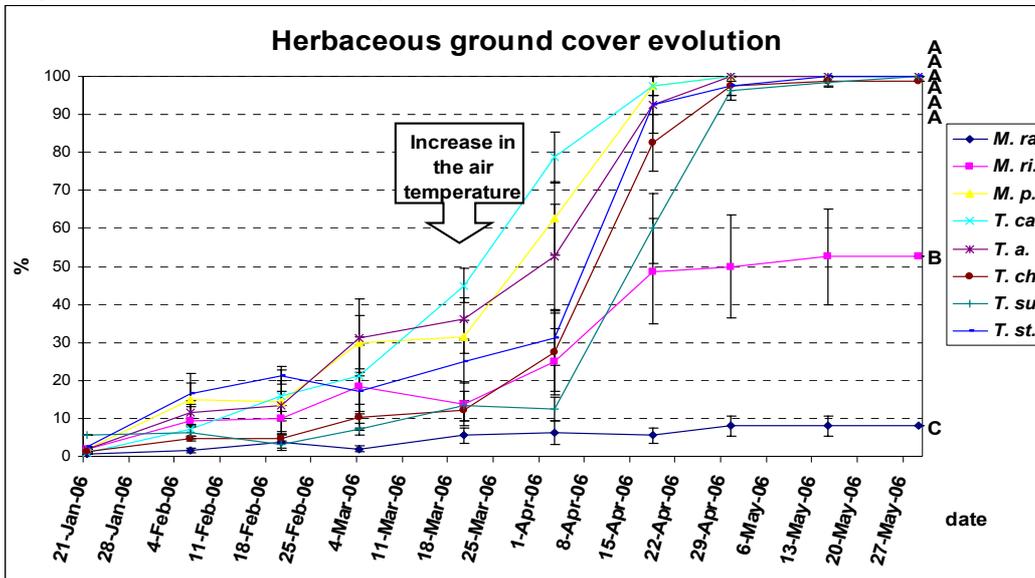
Figure 15 shows a considerable increase in herbaceous ground cover, four months after sowing, due to temperature rising (Figure 5) that was extended over two months to reach stability after maturity stage.

All species reached at the end of the growth cycle the 100 % herbaceous ground cover except *T. ch.* (99 %) *M. ri.* (54 %) and *M. ra.* (8 %) and significant differences were obtained between treatments at the end of the growth cycle, classifying them into three different groups (Figure 15).

Highly significant differences were obtained at the end of the growth cycle between *M. ri.*, *M. ra.* and the other six tested species in which no significant differences were obtained among them (Figure 15).

First species to reach 100 % flowering stage were *T. a.*, *T. ca.* and *M. p.* during the end of April and the last species to reach this stage was *T. su.* after the end of May.

Figure 15: Plant herbaceous ground cover evolution during legume growth cycle

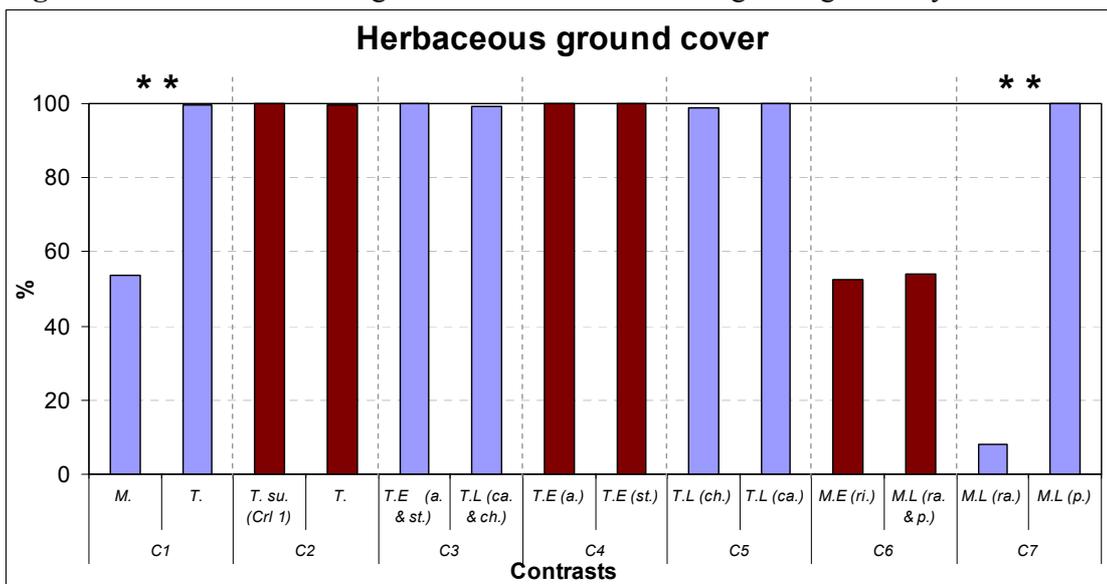


Bars correspond to the standard error and the values that don't have the same letters are different at 1 % (Duncan test).

Same as plant height, herbaceous ground cover was positively highly correlated with air temperature (75 %) and 70 % with rainfall.

Statistical analysis done for the contrasts at the end of the growth cycle (Figure 16) shows highly significant differences between *M.* and *T.* species and also between the two late *M.* accessions (*ra.* and *p.*). This highly significant difference was due to the low herbaceous ground cover percent of *M. ra.* that didn't exceed 10 % at the end of the growth cycle.

Figure 16: Plant herbaceous ground cover at the end of legumes growth cycle



** : Treatments are different at 1 %

3.1.3.3. Flowering dates

Flowering time is among the most responsive or ‘plastic’ of all life history traits to variations in environmental conditions (Ehrman and Cocks, 1996).

Flowering date is an essential parameter when it comes to legumes as it’s the critical time in which the plant fixes the highest quantity of nitrogen (Guet, 1999).

The first species that reached the 100 % flowering stage was *T. a.* with 159 days after sowing (DAS) and the last species was *T. su.* (Cr11) with 174 DAS (Figure 17).

In fact, species were identified as early (E.) or late (L.) species, for contrasts, according to the 100 % flowering stage, as it’s a critical point in which the nitrogen fixation is maximum.

T.E. were *angustifolium* and *stellatum*

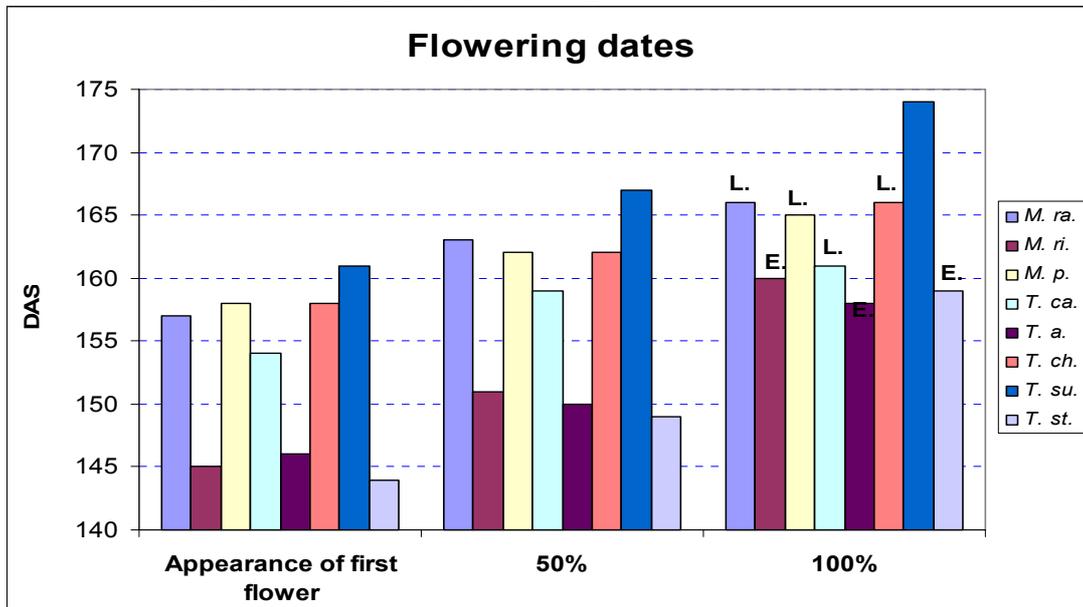
T.L. were *campestre* and *cherleri*

M.E. was *rigidula*

M.L. were *radiata* and *polymorpha*

T. subterraneum was not identified as E. or L. because it’s a control that was used alone in the second contrast, but it was the last one to reach all stages.

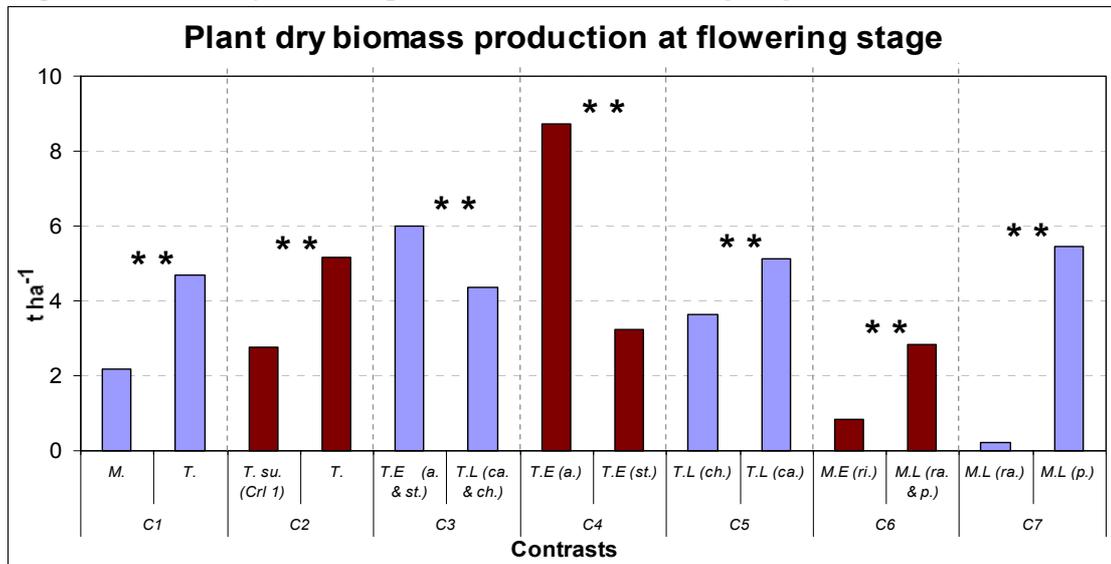
Figure 17: Flowering dates in days after sowing (DAS) at first, 50 and 100 % flowering stages; E.: early species; L.: late species



3.1.3.4. Plant dry biomass production

Plant dry biomass production is an important parameter to take into consideration with legumes especially if legumes will be incorporated after flowering stage for their effect on soil organic matter, soil erosion, soil structure, recycling of nutrients (Donahue, 1998). Statistical analysis showed highly significant differences for all the contrasts (Figure 18). Highest dry biomass weight was produced by *T. a.* with 8.7 t ha⁻¹ then *M. p.* (5.5 t ha⁻¹) and the lowest was *M. ra.* with 0.2 t ha⁻¹.

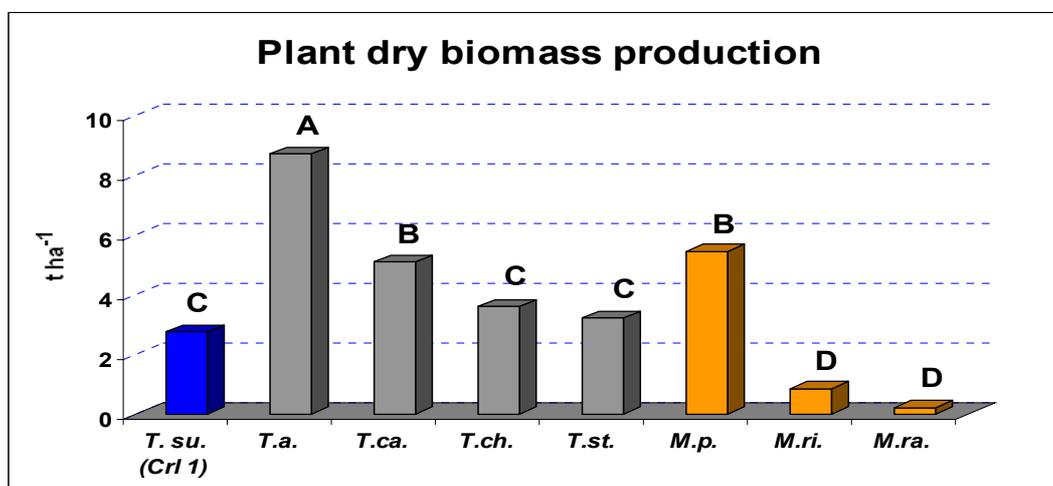
Figure 18: Plant dry biomass production at full-flowering stage



** : Treatments are different at 1 %

High positive correlations were found between dry biomass production and herbaceous ground cover (66 %) and dry biomass production with plant height (61 %).

In order to compare the tested species with the control one (*T. subterraneum*), statistical analysis was done at full-flowering stage and showed four different groups (Figure 19). Highly significant differences were obtained between *T. a.*, *T. ca.*, *M. p.* and *T. su.* (Cr11) in which, the control showed the lower value. Also highly significant differences were observed between the control and *M. ri.* and *M. ra.* in which the control showed a higher value.

Figure 19: Legumes dry biomass production at full-flowering stage

Values that don't have the same letters are different at 1 % (Duncan test).

3.1.3.5. Plant total nitrogen content at flowering stage

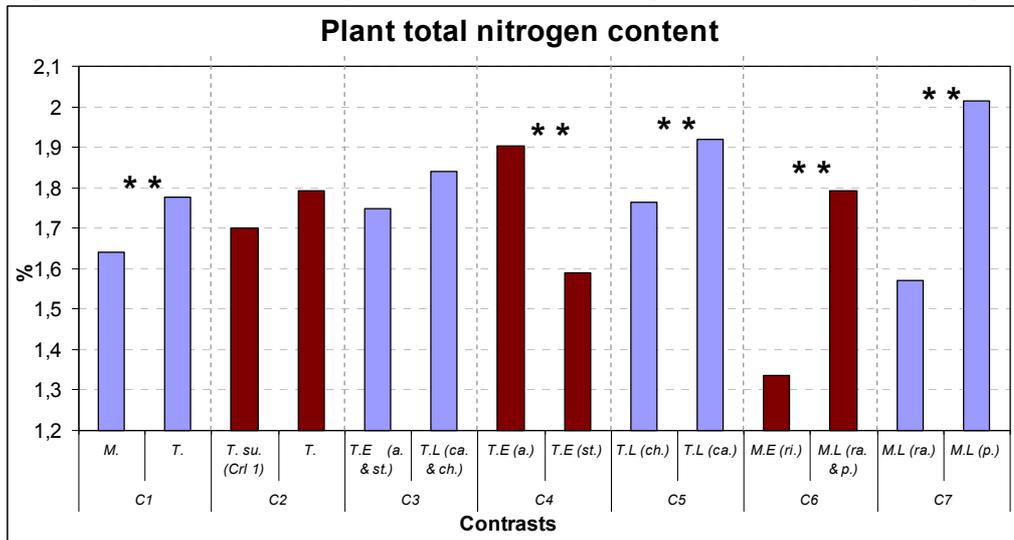
One of the common uses of legumes in organic agriculture is green manure where fresh biomass is incorporated into the soil and nitrogen is slowly released and made available to the subsequent crop. Therefore it's important to know the amount of nitrogen in the aerial plant parts in order to know the fixed nitrogen by the plant using also the biomass production.

High significant differences were obtained (Figure 20) between *Trifolium* (1.78 %) and *Medicago* (1.64 %) species. High significant differences were also observed between the two early (*a.* and *st.*) and late *T.* species (*ch.* and *ca.*), between early and late *M.* species and the two late *M.* species (*ra.* and *p.*).

The highest % of total nitrogen was 2 % in the case of *M. p.*, then *T. ca.* (1.92 %) and the lowest value was 1.90 % by *M. ri.*

Positive high correlation of 68 % was observed between the percentage of total nitrogen and dry biomass production.

Figure 20: Plant total nitrogen content in the aerial parts at full-flowering stage



** : Treatments are different at 1 %

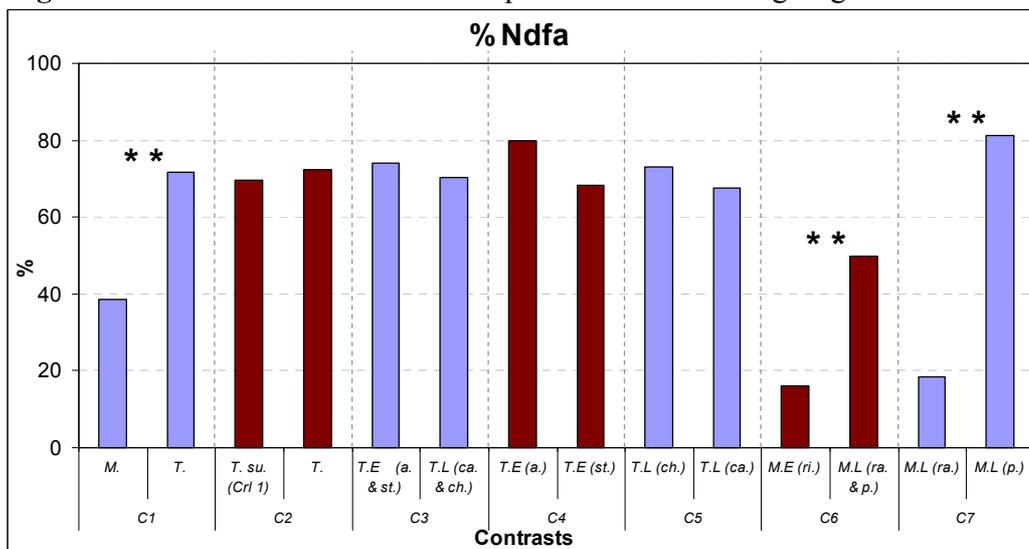
3.1.3.6. Plant nitrogen derived from atmosphere (% Ndfa)

The highest % of Ndfa was 81.5 % reached by *M. p.* and *T. a.* with 80 % and the lowest value was 18 % by *M. ri.* (Figure 21).

Statistical analysis done after full-flowering stage showed highly significant differences between *Medicago* (38 %) and *Trifolium* (72 %) species, between *M.E.* and *M.L.* and between the two late *M. (ra. and p.)* (Figure 21).

High positive correlation was observed between % Ndfa and herbaceous ground cover (84 %) and 74 % with plant height.

Figure 21: Plant N-derived from atmosphere at full-flowering stage



** : Treatments are different at 1 %

The % Ndfa is a parameter that could give an appraisal about the species that will fix the highest amount of nitrogen per hectare. Thus, *M. p.* should fix the highest amount of nitrogen, as 81.5 % of its nitrogen was fixed from the atmosphere than in the second place comes *T. a.* with 80 % Ndfa.

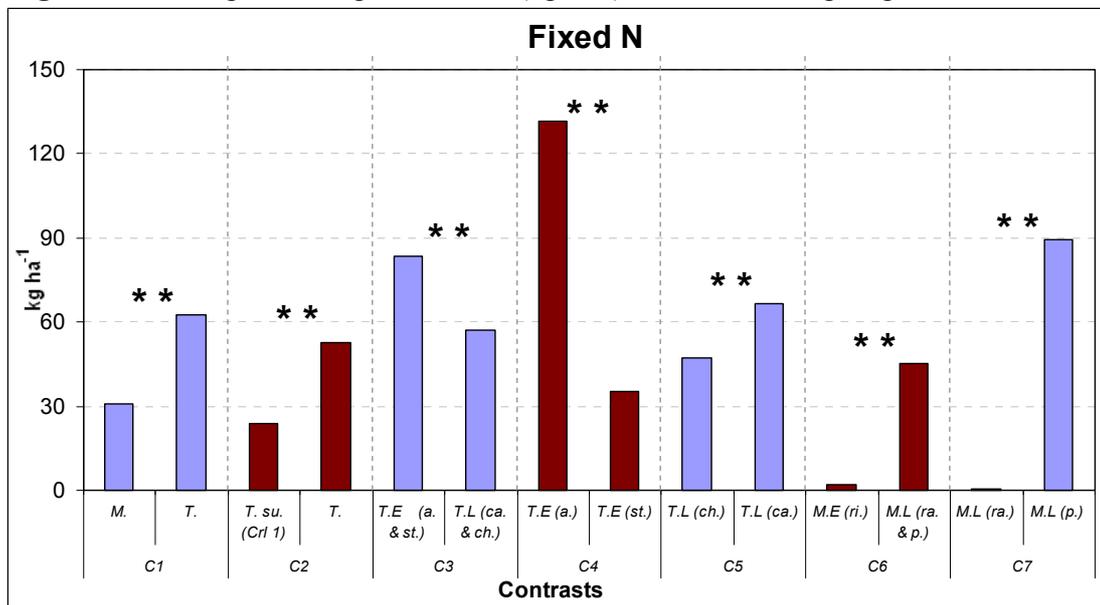
Such appraisal could be supported by the calculation of biologically fixed nitrogen per hectare.

3.1.3.7. Biological nitrogen fixation (BNF)

It's very important for legumes and for organic agriculture to know the fixed amount of nitrogen in kg ha^{-1} . We can choose better the subsequent crop according to the amount of nitrogen that was fixed by each legume species. For annual legumes, the range of fixed nitrogen begins from almost nothing to 56 - 112 kg ha^{-1} (Evers, 2003).

Statistical analysis (Figure 22) shows highly significant differences between all contrasts. In average, *T.* species were better than *M.* species. For *T.* species the highest value was observed by *T. a.* with 132 kg ha^{-1} of fixed nitrogen, then *T. ca.* (67 kg ha^{-1}), *T. ch.* (47 kg ha^{-1}) and *T. st.* (35 kg ha^{-1}). Regarding *M.* species, the highest nitrogen fixation was reached by *M. p.* with 90 kg ha^{-1} then *M. ri.* (2 kg ha^{-1}) and *M. ra.* (0.7 kg ha^{-1}).

Figure 22: Biological nitrogen Fixation (kg ha^{-1}) at full-flowering stage



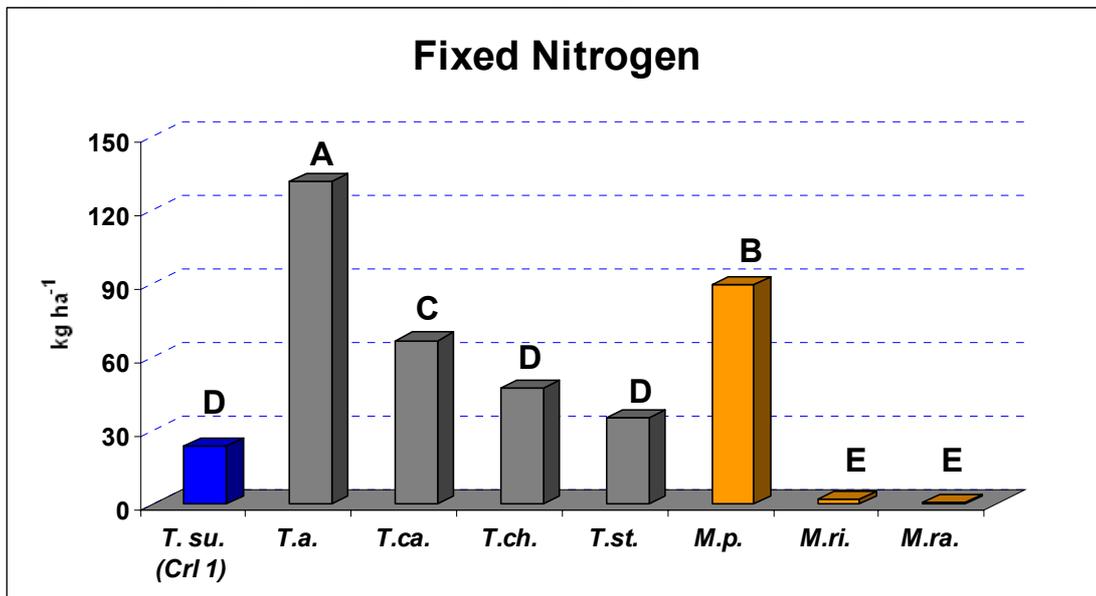
** : Treatments are different at 1 %

The obtained results were not as we predicted in previous Ndfa%, in which *M. p.* would fix the highest amount of nitrogen per hectare (as it showed the highest % Ndfa), because, in fact, *T. a.* fixed 40 kg ha⁻¹ more nitrogen than *M. p.*

This difference was mainly due to the positive high correlation of 98 % that was found between nitrogen fixation and dry biomass production in which *T. a.* showed higher value (8.7 t ha⁻¹) than *M. p.* (5.5 t ha⁻¹).

Statistical analysis was done at full-flowering stage to compare the tested species with the Ctrl 1 (Figure 23). Highly significant differences were obtained between *T. a.*, *M. p.*, *T. ca.* and *T. su.* (Ctrl1), in which, the control showed the lower value. Also highly significant differences were observed between the control and *M. ri.* and *M. ra.* in which the control showed a higher value.

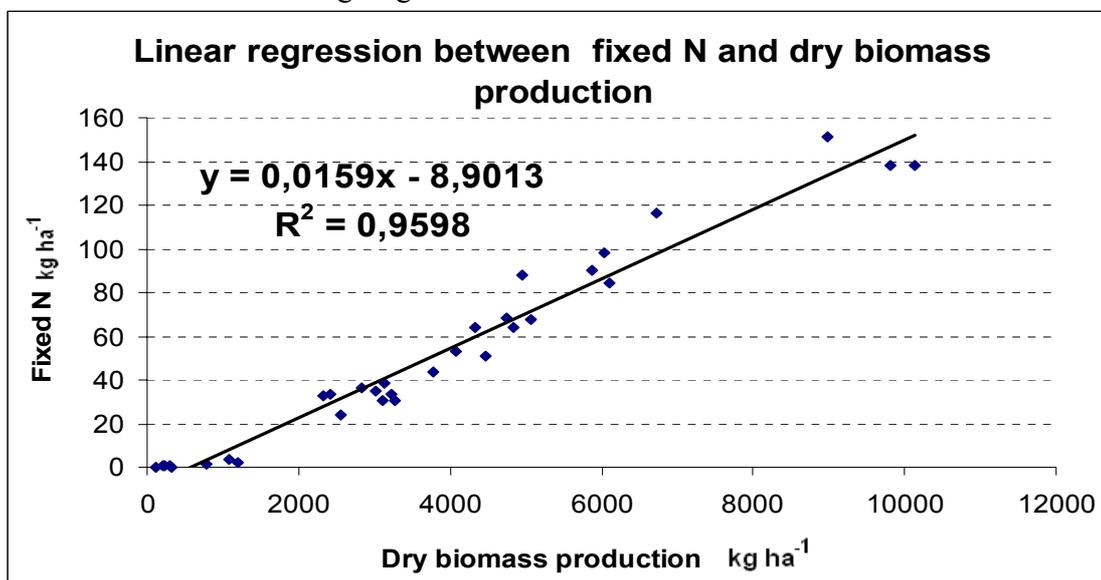
Figure 23: BNF (kg ha⁻¹) after full-flowering stage



Values that don't have the same letters are different at 1 % (Duncan test).

Linear regression was done between fixed nitrogen and dry biomass production (Figure 24), as it's an easy agronomic parameter to measure in the field to predict the amount of fixed nitrogen per hectare.

Figure 24: Linear regression between fixed nitrogen and dry biomass production at full-flowering stage



According to Figure 24 we can predict the fixed nitrogen according to the dry biomass production in which:

$$\text{Fixed nitrogen (kg ha}^{-1}\text{)} = 0.0159 \times \text{Dry biomass production (kg ha}^{-1}\text{)} - 8.9013$$

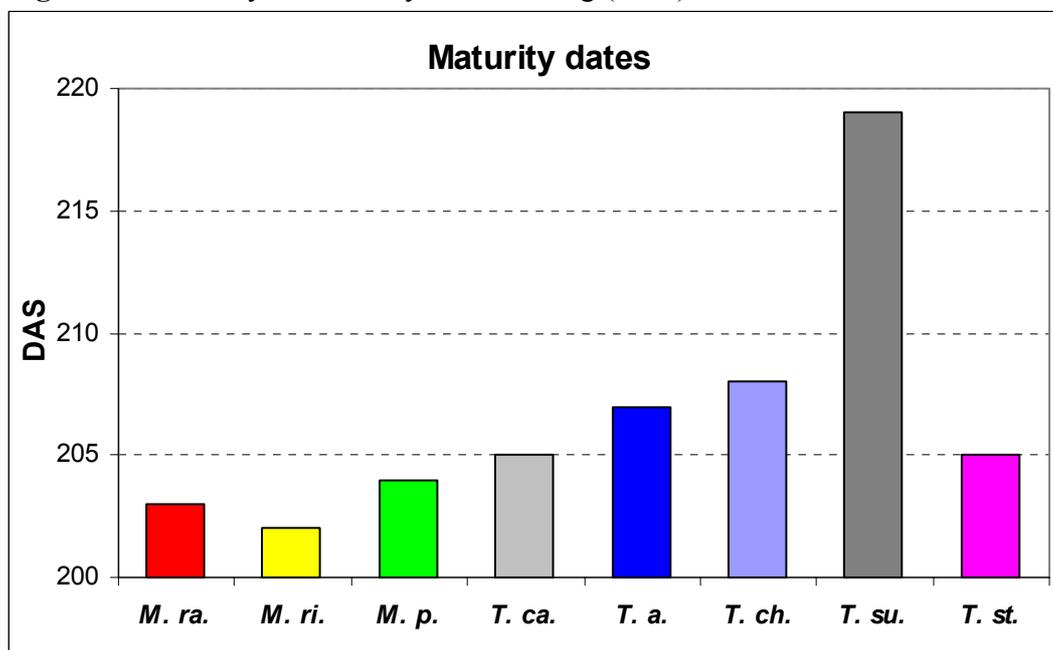
The higher the dry biomass production, the higher the nitrogen fixation is and there is a probability of 96 % to have the right prediction ($R^2 = 0.9598$).

3.1.3.8. Maturity dates

Data concerning the number of days from sowing till full pod maturation shows considerable variations between species (Figure 25).

M. ri. was the first species to reach maturity stage 203 DAS, then *T. a.* 207 DAS, *M. p.* 204 DAS and the last species to reach maturity stage was *T. su.* (Cr11) with 218 DAS.

T. su. (Cr11) is a late variety compared to the other species. All species reached maturity before 208 DAS except *T. su.*; this might be mainly due to the rainfall that reached about 50 mm after 208 DAS and resulted in delaying 10 days the maturity.

Figure 25: Maturity dates in days after sowing (DAS).

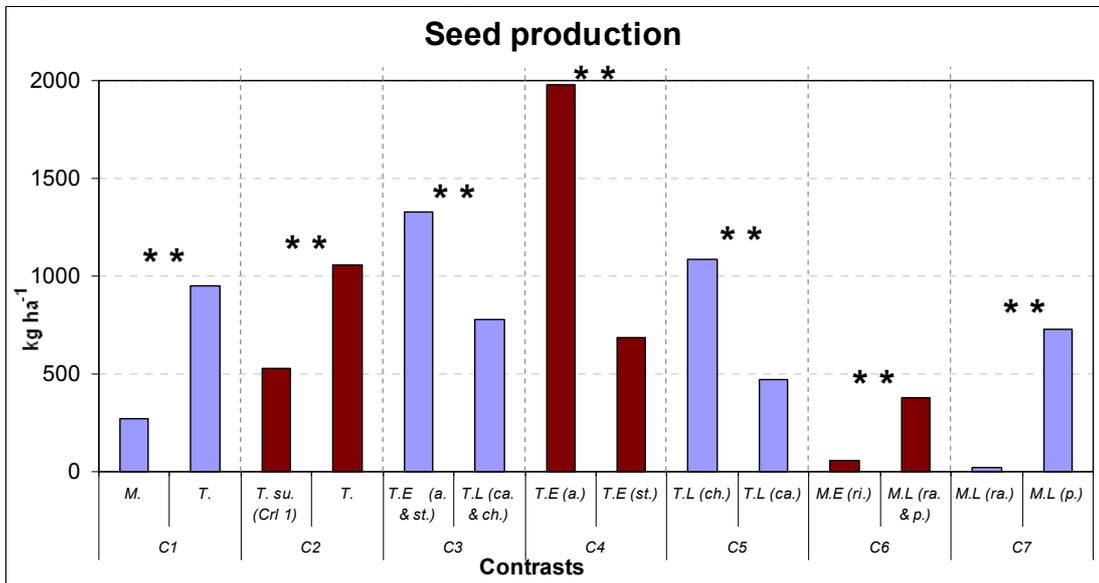
3.1.3.9. Seed production

Seed production is an important parameter for self-reseeding legumes concerning self germination during the next growth cycle without any human intervention or even for seed bank collection.

Statistical analysis done at the end of the growth cycle showed highly significant differences for all contrasts (Figure 26). In average *T.* species produced more seeds than *M.* species.

Concerning *T.* species, *T. a.* showed the highest seed production with 1976 kg ha⁻¹, for *M.* species, the highest value was reached by *M. p.* with 731 kg ha⁻¹ and the lowest species that produced seeds was *M. ra.* with 22 kg ha⁻¹.

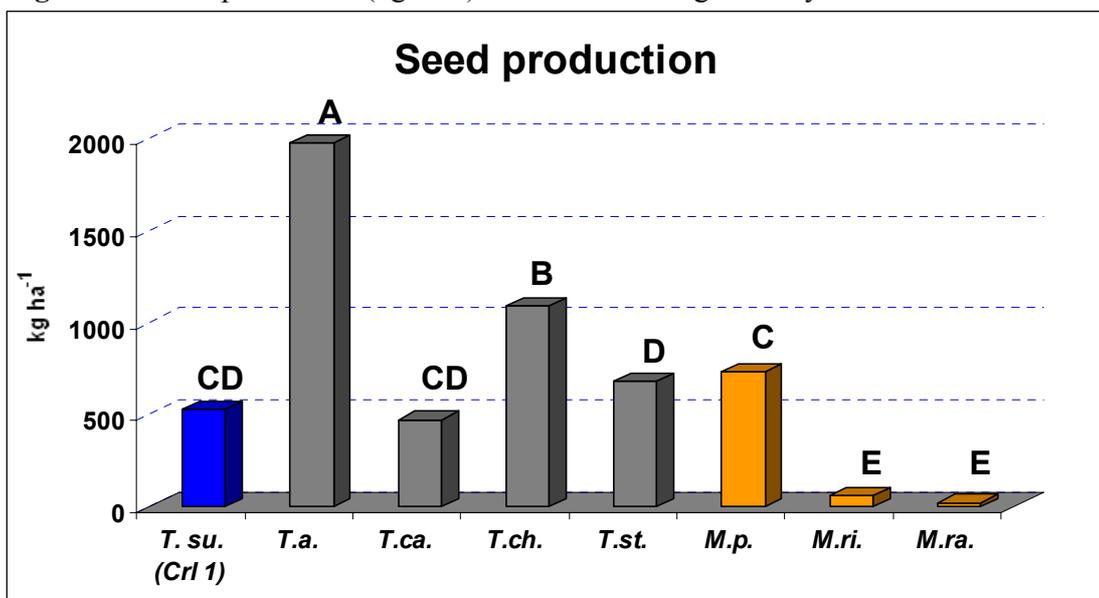
Figure 26: Seed production (kg ha⁻¹) after maturity stage



** : Treatments are different at 1 %

Statistical analysis was done at the end of the growth cycle to compare the tested species with the control 1 (Figure 27) that resulted in 5 different groups. Highly significant differences were obtained between *T. a.*, *T. ch.* and *T. su.* (Cr11) in which the control showed a lower value. Also highly significant differences were observed between the control and *M. ra.* and *M. ri.* in which the control showed a higher value.

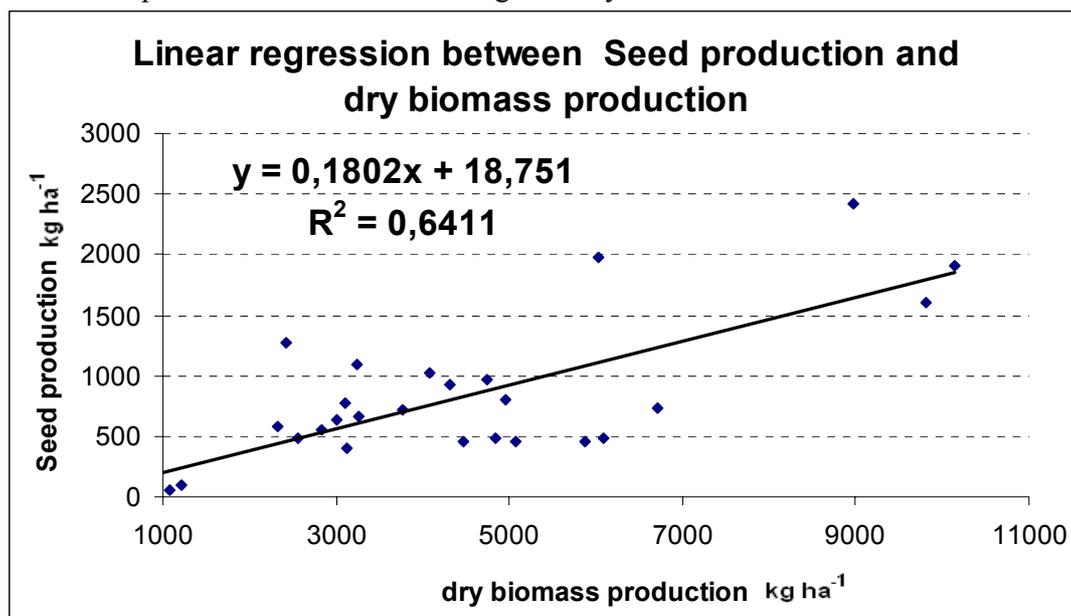
Figure 27: Seed production (kg ha⁻¹) at the end of the growth cycle



Values that don't have the same letters are different at 1 % (Duncan test).

Seed production was highly positively correlated with dry biomass production (80%). Linear regression was done between seed production and dry biomass production (Figure 28), as it's an easy agronomic parameter to measure in the field to predict the amount seed production.

Figure 28: Linear regression between seed production and dry biomass production at the end of the growth cycle



According to Figure 28 we can predict the seed production according to the dry biomass production in which:

$$\text{Seed production (kg ha}^{-1}\text{)} = 0.1802 \times \text{Dry biomass production (kg ha}^{-1}\text{)} + 18.751$$

The higher the dry biomass production, the higher the production of seeds is and there is a probability of 64 % to have the right prediction ($R^2 = 0.64$).

3.1.4. Soil Relative Humidity after full-flowering stage

Soil relative humidity (SRH) showed highly significant differences between *Medicago* and *Trifolium* species. Significant differences were found between *T. su.* and the other four *T.* species, between early and late *T.* species, between the two late *T.* (*ch. and ca.*) and the significant difference was also observed between the N-pool compared to all the other plots (Figure 29).

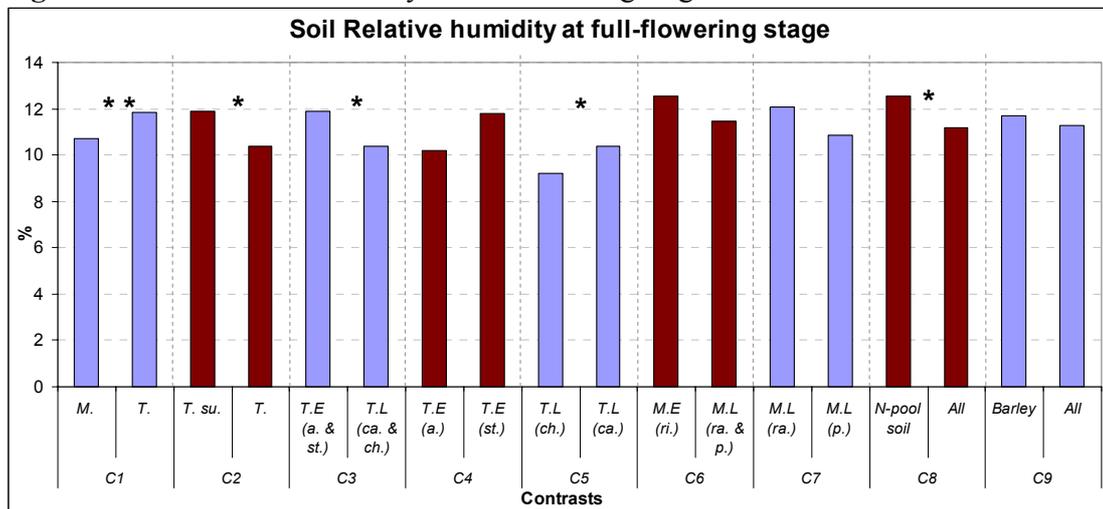
In this experiment the SRH of N-pool plots (Cr14: not cultivated and weeds were always taken out during the experiment) was higher than the average of all the other plots, in fact this result should be inverted because according to Campiglia (1999), legumes have an effect in decreasing evaporation especially for their herbaceous ground cover effect, as

legume are known to preserve SRH. This fact is probably due to the rainfall that was available during the whole cycle (Figure 5). If there was no rainfall at that time, SRH of N-pool plot should have been lower than the plots occupied by legumes as evaporation would have been higher.

M. species were able to decrease soil relative humidity more than *T.* species, even though *T.* species had more plant height and herbaceous ground cover. This could be explained by assuming that *M.* species could uptake more water than *T.* species, same results were found by Chatterton and Chatterton (1996).

The highest SRH was found in the case of *M. ri* with 12.54 %. For *T.* species, the highest value was reached by *T. su.* with 11.91 % and the lowest SRH was found in the case of *T. ch.* with 9.20 % (Figure 29).

Figure 29: Soil relative humidity at full-flowering stage



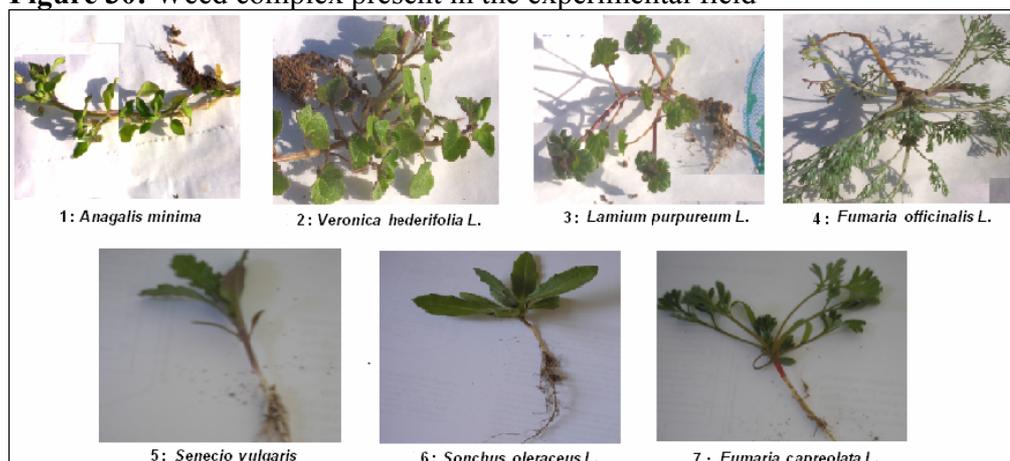
*: Treatments are different at 5 % and **: Treatments are different at 1 %.

3.1.5. Weed occurrence and disease incidence

3.1.5.1. Weed occurrence

Weeds found in the experimental field were seven (Figure 30): *Anagalis minima*, *Veronica hederifolia* L., *Lamium purpureum* L., *Fumaria officinalis* L., *Senecio vulgaris*, *Sonchus oleraceus* L., *Fumaria capreolata* L. (Viggiani, 1990).

Figure 30: Weed complex present in the experimental field



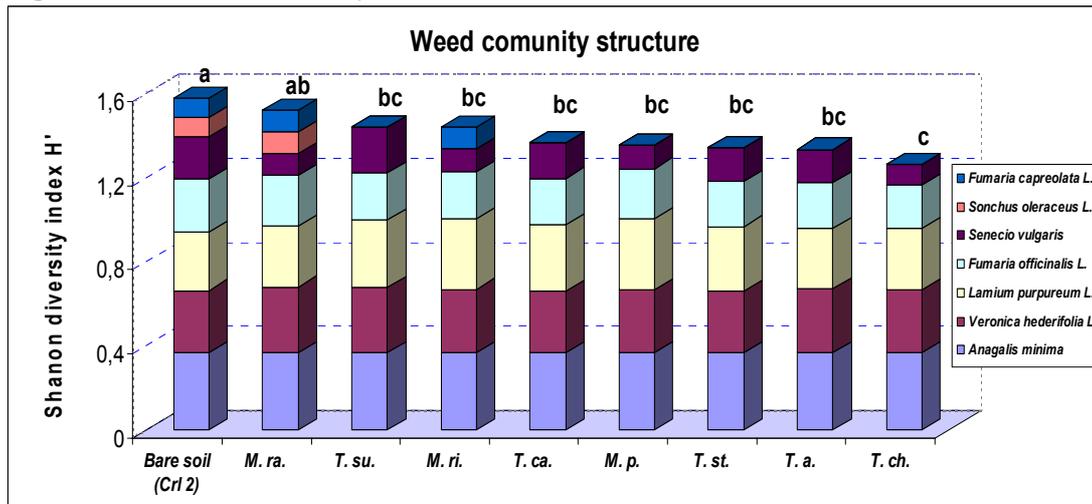
Field biodiversity (Table 9) concerning weeds was quite high with a Shannon diversity index "H" equal to 0.87 but according to the Shannon equitability index "E" which was 0.45, we can realise that the weeds were not well distributed, so we have high number of *Anagalis minima* (about 400 plants m⁻²) compared to the other weed species that were found in small quantities (about 30 plants m⁻²).

Table 9: Field biodiversity identification using Shannon Diversity Index "H" and Shannon Equitability Index "E"

	Shannon Diversity Index "H"	Shannon Equitability Index "E"
<i>T. subterraneum</i>	1,04	0,54
Bare soil	0,93	0,48
<i>M. radiata</i>	0,90	0,46
<i>M. rigidula</i>	0,85	0,44
<i>M. polymorpha</i>	0,84	0,43
<i>T. campestre</i>	0,84	0,43
<i>T. angustifolium</i>	0,84	0,43
<i>T. stellatum</i>	0,82	0,42
<i>T. cherleri</i>	0,77	0,39
Field Biodiversity	0,87	0,45

Figure 31 shows the weed community structure taking into consideration the Shannon diversity index of each weed species in each plot. We can conclude that bare soil plots (Cr1. 2) contained the highest number of weeds ($H' = 1.58$) and showed a significant difference compared with all the other plots except *M. ra.* ($H' = 1.52$). Whereas the lowest weed community structure was found with *T. ch.* ($H' = 1.26$) plots. This significant difference could be explained by the absence of legumes in the bare soil plots (Cr12), so there was a low effect of legumes on the weeds.

Figure 31: Weed community structure



Values that don't have the same letters are different at 5 % (Duncan test).

In fact, these results show the presence of weeds in the experimental field at the beginning of legume cycle but the effect of legume species on different weeds species, may be observed during the next growth cycles.

3.1.5.2. Disease incidence

One month and a half before the end of the growth cycle (end of April), the experimental field was infested by *Pseudopeziza medicaginis* an ascomycete fungi belonging to the family of the dermataceae.

In fact, as reported by Brygoo (2001), *Pseudopeziza medicaginis* attacks especially *M.* species and between the three *M.* species that we have only *M. p.* was attacked at the lower part of the plant. The attack was not strong and *M. p.* plants were at pod stage before maturity. However *Pseudopeziza medicaginis* didn't attack pods, only leaves were attacked

Furthermore, we noticed that all other species were completely immune to this infection and showed to be highly resistant.

3.1.6. Global evaluation of the tested species during the first cropping cycle

In order to make a global evaluation of legume species performances, the parameters tested are illustrated in table 10. In order to make possible ranking of cultivars, each of them was assigned a value from 1 to 5, in which 5 refer to the maximum value assigned to a certain species and 1 refers to the lowest. The sum of all values gives the total value that species are given and the rank.

Table 10: Ranking of evaluated species according to the agronomic performances

Rank		Germination Test %	Early Flowering %	Early Maturity DAS	Herbaceous ground cover %	Dry biomass Production t ha ⁻¹	Plant height cm	Weight of Seeds g	% Ndfa	Fixed N kg ha ⁻¹	Sum over 45
1	<i>T. angustifolium</i>	5	5	4	5	5	4	5	5	5	43
2	<i>M. polymorpha</i>	5	3	5	5	4	5	1	5	4	37
3	<i>T. stellatum</i>	5	5	5	5	2	3	2	5	2	34
4	<i>T. campestre</i>	1	5	5	5	3	4	2	4	3	32
5	<i>T. cheleri</i>	4	3	4	5	2	3	3	5	2	31
6	<i>T. subteraneum</i> (Cr11)	3	1	1	5	2	5	2	5	2	26
7	<i>M. rigidula</i>	4	5	5	3	1	2	1	1	1	23
8	<i>M. radiata</i>	1	3	5	1	1	1	1	1	1	15

5 corresponds to the maximum value and 1 to the lowest

In terms of agronomic performance, *T.* species were on average more and better adapted to the pedo-climatic conditions of the experimental site.

Regarding the genus *Trifolium.*, *T. angustifolium* showed to be a very promising one compared to all the other species and for the genus *Medicago*, *M. polymorpha* is considered as a promising one.

M. radiata and *M. rigidula* showed the lowest values regarding most of the tested parameters.

3.2. Legumes self-establishment and BNF

3.2.1. Climatic data

3.2.1.1. Climatic conditions during summer 2006

Climatic data were also recorded during summer period of 2006 in order to analyse the effect of summer temperatures and precipitation on legumes seeds left in the field at the end of the last season (Figure 5 and 6).

Temperature was quite constant during the period between early June and third week of July. It started to increase slightly to reach its peak that fluctuated between 34°C as the maximum and 23°C as the minimum temperature in the 4th of August. After that, temperature started to decrease gradually until the end of September in which maximum and minimum temperatures were 26°C and 16°C respectively.

Late in June, 19 mm of precipitation occurred followed by dry July in which very small amounts of precipitation took place until early August where 58 mm were recorded and again, very little precipitation took place until late September with 100 mm. Those data show an important alternation of drought-rainfall which could influence the dormancy and viability of seeds left in the field by inducing a false breaks of seeds dormancy and therefore, massive losses of germinated seedlings.

3.2.1.2. Climatic conditions during legumes growth cycle

Climatic data during legumes growth cycle showed that after germination of legumes (Late in October 2006) there was a gradual decrease in both maximum and minimum temperatures till early January then, temperature started to increase gradually till the end of March (Figure 6).

In the period following the germination stage, temperature fluctuated generally between a maximum of 22.3 °C during late October and a minimum of 3.8 °C during early January. During the whole cycle, except last week of October during germination stage, temperature did not even reach 20 °C while the optimum temperature needed for successful legume growth ranges between 20–22 °C (Covell *et al.*, 1986).

Legumes growth cycle (27.10.2006 – 30.03.2007) was characterized by low precipitations (300 mm) when compared with previous years. The first two months were almost dry and a small quantities of rain occurred, this could cause on one hand a false breaks of the remaining dormant seeds left in the field of some leguminous species and on the other hand, a death of already germinated plants that could not meet their water requirement from the dry soil.

In fact, both low temperature and low precipitation during the first two months of legumes growth cycle affected negatively the growth and development of plants. Starting

from early February in which temperature started to increase and almost continuous reasonable precipitation occurred, a development in plants behaviour was observed in the field.

3.2.1.3. Climatic conditions during zucchini growth cycle

The aim of presenting climatic data during this period is to analyze their effect on zucchini established following legumes incorporation in addition to see their effect on nitrogen level in the soil.

There was a gradual increase in both maximum and minimum temperature during the whole crop cycle. Average temperature fluctuated between 16 and 27 °C. Maximum temperature reached 33.6°C only during the last week of zucchini cycle while minimum temperature was above 10°C during the whole cycle (Figure 6). Such temperature conditions coincided with optimum ones for zucchini crop as it is a warm-season crop and its optimal growing temperature range is 18°C–24°C (Molinar *et al.*, 2000).

Total of 149.6 mm of rain were recorded during the whole cycle, the majority occurred during the beginning of May which could induce nitrogen leaching even in periods with slight quantity of precipitation.

3.2.1.4. Climatic conditions during lettuce growth cycle

During the lettuce growth cycle (late spring-summer 2007) the season was characterized by the greatest temperature, photoperiod, and sunshine duration.

There was quite a constant trend in both maximum and minimum temperature during the whole crop cycle. Average temperature fluctuated between 25 and 27 °C. Maximum temperature reached 34°C only during the last week of lettuce growth cycle while minimum temperature was above 18°C during the whole cycle (Figure 6). Such temperature conditions coincided with optimum ones for lettuce crop as it is a warm-season crop and its optimal growing temperature range is 20°C–24°C (Bianco, 1990).

No precipitation were recorded during the whole lettuce growth cycle. The need of water was ensured through drip Irrigation system.

3.2.2. Self-establishment in the field and site adaptability of legumes

Self-establishment of the leguminous species is an indicator of their persistence which is an important attribute of forage legumes. In absence of persistence, all other measures of success of legumes such as productivity, quality, etc., are meaningless. Persistence is influenced by climate, management, pests and by their interrelations in a given environment (Sulas *et al.*, 2000).

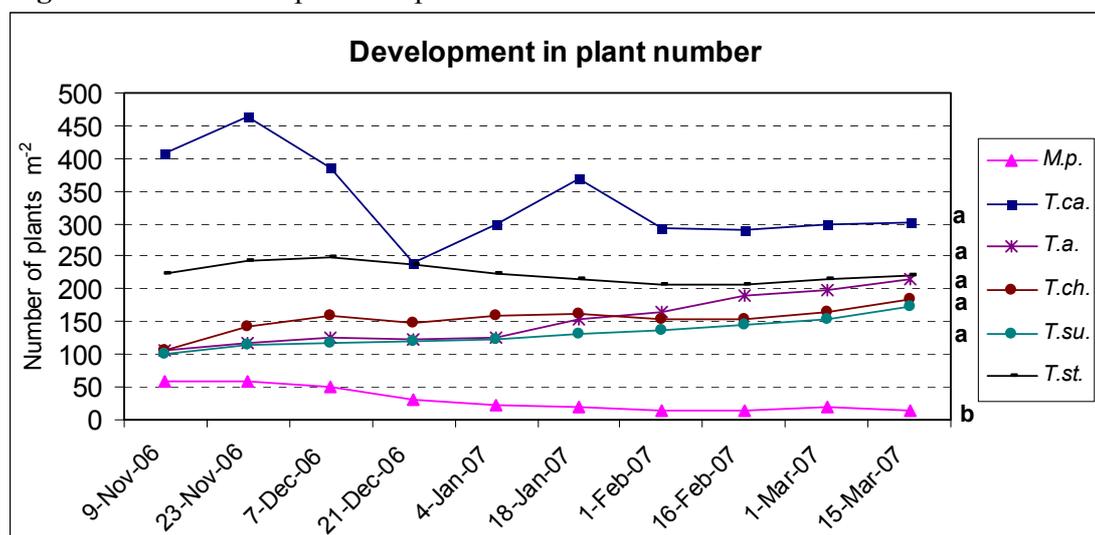
3.2.2.1. Plant number

Not all leguminous species regenerated in the second cropping cycle. This was the case of *M. ra.* and *M. ri.* in addition to very poor re-generation of *M. p.* Similar results were obtained by Cocks (1993) in which very low percentage of *M. p.* seedlings (4-8%) were survived in the first year of regeneration within a research conducted for four years.

On the contrary, Abd El-Moneim and Cocks (1986) who carried out a research concerning the adaptation of *M. ri.* (native to Syria) to a cereal-pasture rotation in north-west Syria, reported that *M. ri.* can regenerate naturally and form productive pastures in rotation with wheat.

Statistical analysis was done for the number of germinated plants in the period just before green manuring. Significant differences were obtained between *M. p.* and all other tested *Trifolium* spp. in which no significant differences were obtained among them (Figure 32).

Figure 32: The development of plants number



Means with the same letter are not significantly different at 5 % (Duncan test).

A reduction in the plants number of *M. p.* can be seen throughout the season. In fact, germinated seedlings were unable to survive due to the attack of *Pseudopeziz medicaginis*; an ascomycete fungi belonging to the family Dermateaceae. The same fungi

attacked *M. p.* last season but one month and a half before the end of the growth cycle. During this season, the fungi attacked the new seedlings and caused dryness followed by rapid death. The fungus could also attack seeds and affect negatively their germination process.

Sulas *et al.*, (2000) reported that, the resistance to fungi is one of the important factors that can affect the legume persistence. According to this, *M.p.* is of a low persistence to the given conditions.

According to the first legumes cropping cycle, both *M. ra.* and *M. ri.* had the lowest seed production at the end of the crop cycle. This could be a reason that reduced their opportunity to germinate during this season in comparison with other species.

The absence of germination in *M. ra.* and *M. ri.*, and the poor re-generation of *M. p.* could also be explained by the unsuitable summer climatic conditions. A substantial proportion of the soft seeds might germinate after summer rain and die in the subsequent drought. Although this was not the case in a study done by Cocks (1993) in which all medics tested in the study (including *M. ri.* and *M. p.*) remained hard until the end of summer, this hypothesis can not be excluded from the present study. On the other hand, seeds of medics could be killed by high summer temperature as it was reported by Abd El-Moneim and Cocks (1986) in which the second author observed that many seeds of 14 *Medicago* spp. were killed by high summer temperature in northern Iraq. In the case of the present study, no observation for seedlings emergence in the field was done during summer period, so those hypotheses could not be tested.

Another explanation could be that seeds resisted germination because of their hard seeds. In fact, a percentage of *Medicago* spp. seeds were observed during the growth cycle on the soil surface unable to germinate. Quinlivan (1971) reported that most legumes produce hard seeds that resist germination for a period of several years, the annual medics show even a greater hardseediness with up to 90% of new seeds (Taylor and Ewing, 1988). But this percentage starts to decrease as the seeds become older if left in field for several years. Same results were obtained by Cocks (1993) in which the rate of hard seeds breakdown of some medics (Including *M.ri.* and *M. p.*) was increasing with age of seeds. The research was conducted during the period (1987-1990) the percentage of hard seeds breakdown was common to all Medics accessions as follow: 9% in 1987, 26% in 1988, 27% in 1989 and 52% in 1990. The longevity of these medics contrasts sharply with that of *T. su.* in which the breakdown of hard seeds is greatest in the first year (Taylor, 1984).

According to these results, the behaviour of *Medicago* spp. in the field could be explained by supposing that it could change positively if seeds are left in the soil for many years, if so -though this is not going with the purpose of green manuring where seeds should have a high germination percentage in the first year in order to be incorporated for subsequent crop. Those annual medics could be exploited for other purposes such as sustaining pastures after several years of bad management or environment-induced poor seed set, or after two or more successive years of cereals.

Moreover, little precipitation took place in the early period of legumes growth cycle (Figure 6). According to Cocks (1993) this little precipitation is considered to be a risk in that drought after early rains will cause the death of newly germinated seedlings. Cocks (1993) also reported that the dry autumn resulted in the death of many seedlings of medics including *M. p.* while seeds that did not emerge were presumed to have germinated though not necessarily emerged due to lack of enough water in the soil.

Additionally, according to Cocks (1993), seeds of medics remain on the soil surface and the loss of seeds is usually greater than for subterranean clover that burr its seeds. Burial limits the exposure of the seeds to diurnal fluctuations in temperature (Taylor, 1984). Seed burial probably stimulates germination in the first year through improved seed/soil contact. Usually, the soil surface dries out a few days after rain. Seeds are protected from rapid wetting by pod walls and spines and therefore do not imbibe sufficient water to germinate before the soil dries out. At depth greater than about 5 cm the soil remains wet for much longer and germination proceeds. Imbibitions by some seeds may also have been slow because cracks in the newly softened seeds are small and there are a longer than normal need for good seed/soil contact before germination began, again, this would be more likely to occur with buried pods than with pods laying on the soil surface. In fact, according to the research conducted by Cocks (1993), the germination of buried seeds was better than seeds on or near the surface in the first year of regeneration. This is with agreement with results obtained by Sulas *et al.*, (2000) who reported that the better buried burrs produce more viable seeds.

According to this result, the behaviour of *T. su.*, *T. a.* and the rest of *Trifolium* spp. in comparison with *Medicago* spp. can be explained since their seeds were buried in the soil while seeds of medics were observed on the soil surface. However, there may have been another risk that caused seeds losses in medics, for example resulting from seed harvesting ants and other insects due to the presence of seeds on soil surface.

According to Taylor and Ewing (1988), floral peduncles are strong and thick in *T. su.* which help to bury its burrs so, the success of *T. su.* is linked to its ability to bury pods, escaping predation by insects and the severe environmental effects on seed bank size (Rossiter *et al.*, 1985). Floral peduncles are also very strong in *T. a.*, so it could have the same advantage of *T. su.* by having buried burrs. In fact, *T. su.* and *T. a.* self-established very well, this will be more clear from the data related to their ground cover percentage and plant height that will come next.

Quinlivan (1971) described *T. su.* as producing seeds that have a summer dormancy that enable seeds to escape from severe summer conditions. According to Fairbrother and Rowe (1995), Seeds of subterranean clover exhibit: (i) an embryo dormancy due to an immature embryo and (ii) a physiological dormancy, due to unsuitable ambient temperatures, that prevent germination after sporadic summer rains that would be insufficient to support the growth of seedlings, and prior to the formation of an impermeable seed coat.

In general, inhibition of germination at high temperatures during summer period occurs in *Trifolium* spp. due to the maintenance of dormancy while germination can continue at lower temperatures. Another mechanism is slow germination until effective soil moisture is assured (Cocks, 1997). This can explain the slow development in number of plants in most of *Trifolium* spp. including *T. st.* and *T. ch.*

T. ca. showed different behaviour from all other species since its germination was the most affected by climatic conditions. Germination took place following the poor rain periods of October but there was a clear decrease in the number of germinated seedlings that died off, this could be due to the very low precipitation coupled with the decrease in temperature during that period (Figure 32). Number of plants started to increase early in January where a clear increase in precipitation and a gradual increase in temperature were observed. Again, mortality took place until early February due to the decrease in precipitation. Following that period, number of plants started to increase and finally reach some kind of stability during March where enough amounts of rain occurred.

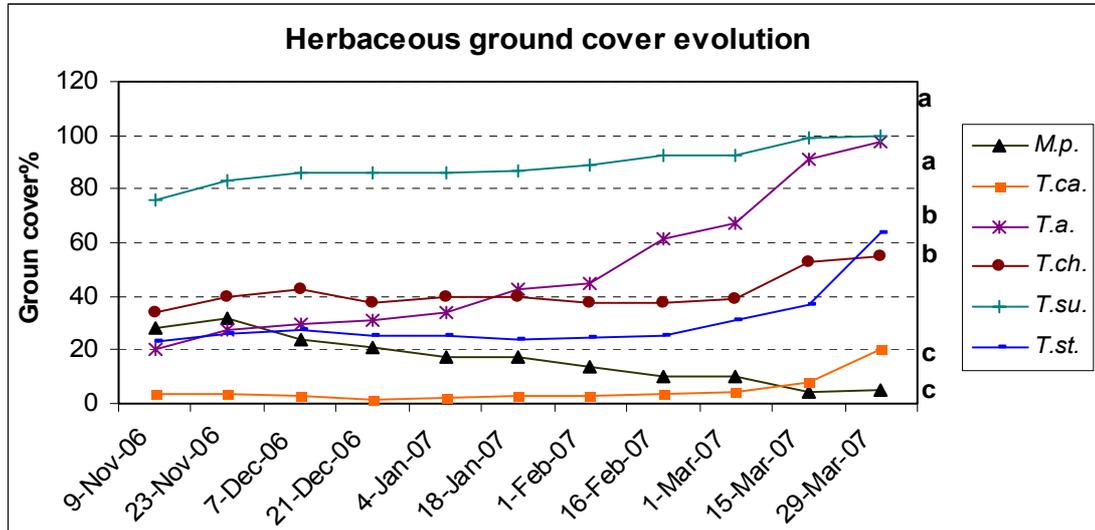
It should be taken in consideration that although *T. ca.* achieved the highest number of plants at the period just before green manuring (300 plants per m²), it was not the indicator to consider it as the best performing species. This can be clearly seen from the data related to ground cover percentage and plant height which will be presented next.

3.2.2.2. Herbaceous ground cover

Herbaceous ground cover is an important parameter to evaluate species persistence. Plant ground cover could assure weed competition, soil protection from erosion and water loss, optimization of nutrient cycle and increase of organic matter in soil, hence, increasing the crop yield of the subsequent crop (Campiglia, 1999).

Among tested species, *T. a.* reached the highest ground cover percentage 97.5%. The lowest percentage was 4.75% by *M. p.* Statistical analysis was done in the period just before incorporation, three homogeneous groups were resulted (Figure 33).

Figure 33: Plants ground cover evolution until the period just before legumes incorporation



Means with the same letter are not significantly different at 5 % (Duncan test)

Although *T. a.* started with relatively low ground cover percentage early in the season, it could increase in very fast rate in comparison with the others finally to reach value not significantly different from the standard control (*T. su.*) while the rest of the tested species were significantly lower than it.

The herbaceous ground cover of *T. ca.* was the lowest during the whole cycle. It started to increase in the middle of March, even higher than *M. p.* This is due to enough precipitation took place during that period which enabled on one hand germinated plants to survive and remaining seeds in the field to germinate on the other hand.

The increase in ground cover in both *T. st.* and *T. ch.* was gradual though a clear increase in the middle of March was observed. Again, this is due to the sufficient precipitation that took place during that period.

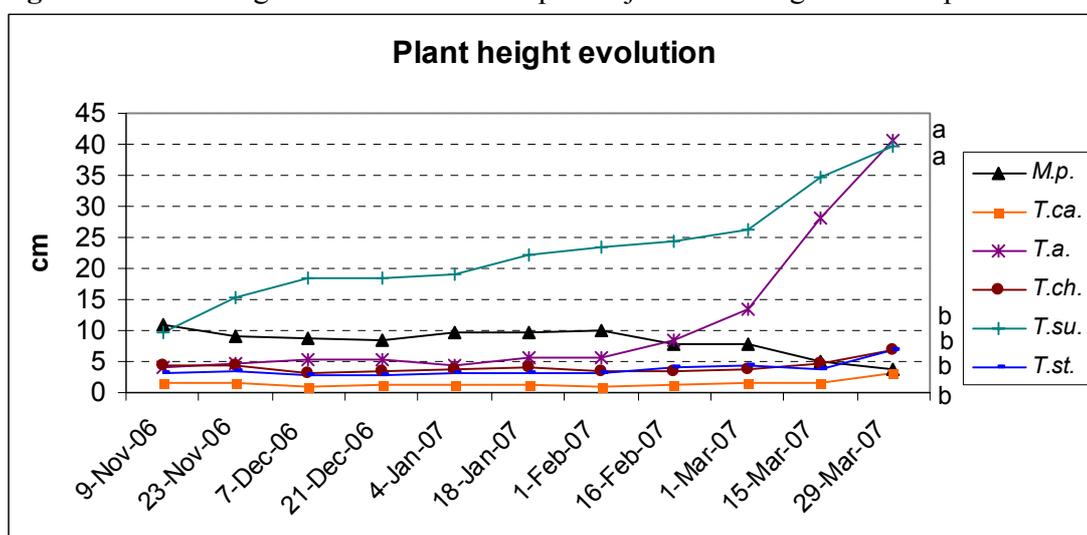
The reduction in the herbaceous ground cover of *M. p.* is due to the attack of the fungus *pseudopeziz medicaginis*, mentioned before, which resulted in the dryness of plants leaves and tips, consequently, reducing their soil coverage.

3.2.2.3. Plant height

No clear increase in plant height was observed in all tested species except *T. a.* which started to increase during the beginning of March.

Statistical analysis was done in the period just before incorporation; *T.a.* was not significantly different from the standard control (*T. su.*). The rest of the tested species were significantly different from the standard control in which they showed lower values with no significant differences among them (Figure 34).

Figure 34: Plant height evolution until the period just before legumes incorporation



Means with the same letter are not significantly different at 5 % (Duncan test)

T. a. started with values similar to other species but it could increase very fast starting from early February to reach the highest value (40.58 cm) in the period just before incorporation.

According to the last year results, maximum plant height was reached by *M. p.* with 56 cm and it was even higher than maximum value (30 cm) reported by Zohary (1987) and (50 cm) reported by Lonchamp (2000). In this year, *M. p.* achieved only 3.75 cm though it started with 10.83 cm at the beginning which was similar to the standard control, this is again due to the attack of the fungus *pseudopeziz medicaginis* that attacked plants tips causing deterioration, hence, reduction in their height.

Plant height of *T. st.*, *T. ch.* and *T. ca.* was almost constant during the whole season. A slight increase was observed in mid March when soil water content increased due to enough precipitation. This is similar to the trend of herbaceous ground cover development.

3.2.2.4. Legumes plants dry biomass production

Plant dry biomass production is an important parameter to be taken into consideration with legumes, especially if legumes will be incorporated for their effect on soil organic matter, soil erosion, soil structure and recycling nutrients in order to provide better conditions for subsequent crop growth (Donahue, 1998).

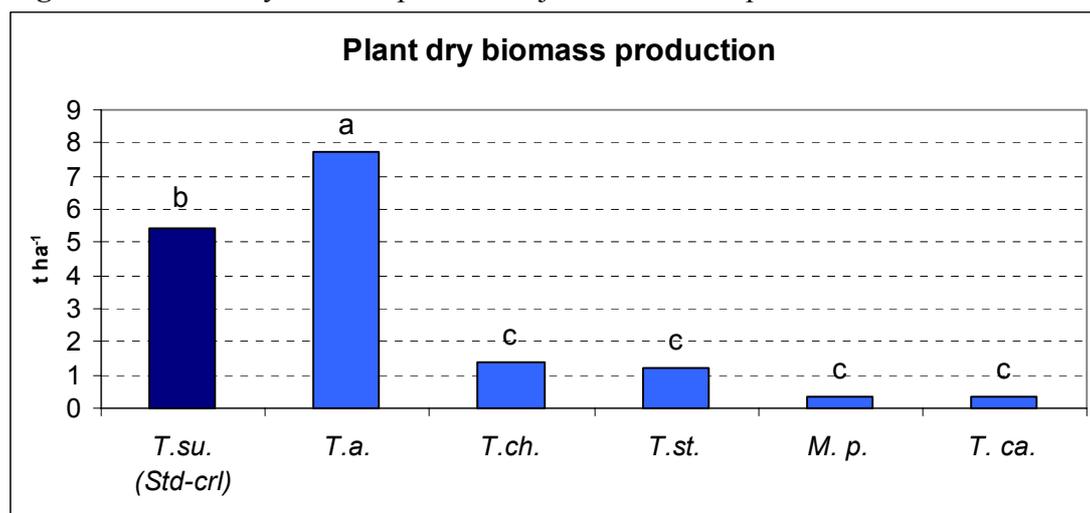
Ashraf *et al.*, (2004) reported that a green manure crops with higher potential to accumulate biomass might have an additional advantages assuming that the amounts of both organic carbon and nitrogen may play an important role in the beneficial effects of green manures.

The highest dry biomass produced was 7.74 t ha⁻¹ by *T. a.* and the lowest was by *T.ca.* and *M. p.* with 0.32 t ha⁻¹ and 0.33 t ha⁻¹ respectively.

In order to compare the tested species with the standard control (*T. su.*), statistical analysis was done for produced dry biomass just before green manuring and showed three homogenous groups (Figure 35).

Highly significant differences were obtained between *T. a.* and the standard control (*T. su.*) in which the control showed a lower value. Also highly significant differences were observed between the control and the rest of the tested species in which the control showed a higher value. A high positive correlations were found between dry biomass production and herbaceous ground cover (72%) and dry biomass production and plant height (91%).

Figure 35: Plants dry biomass production just before incorporation



Means with the same letter are not significantly different at 5% (Duncan test)

Similar behaviour of *T. a.* was observed last year in which it produced the highest dry biomass (8.7 t ha⁻¹). In general, all tested species had last year higher dry biomass compared to this year. Values of last year dry biomass were 5.5, 5.4, 3.5 and 3.4 t ha⁻¹ for *M. p.*, *T. ca.*, *T. ch.* and *T. st.* respectively.

Additionally, *T. ca.* and *M. p.* achieved higher values than the standard control (*T. su.*) and *T. c.* and *T.s.* achieved values similar to it during the last season, while in this season this was not the case, since all of them achieved lower values than the standard control. So, only *T. a.* is still in the same trend of last year by having value higher than the standard control.

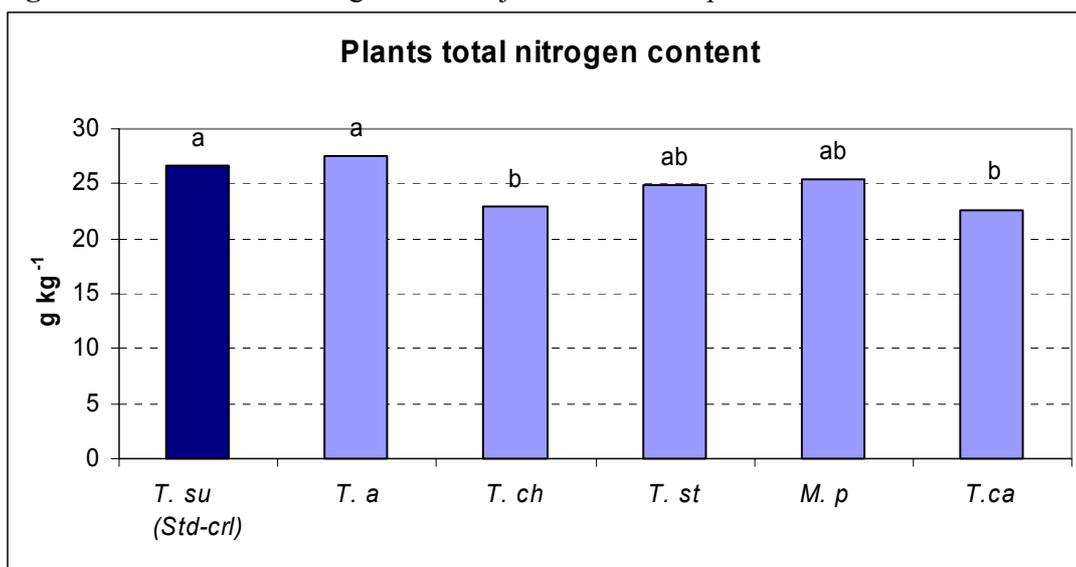
Similar results were obtained by Jamea (2004) who reported that all tested legumes species in their second year had lower dry biomass than the first year when they were established.

3.2.2.4. Plants total nitrogen content

Nitrogen in plant tissues exists in complex organic compounds, proteins and chlorophyll. However, it must be present as ammonium or nitrate ions in soil in order for plants to absorb it. After incorporation of the cover crops, the plant material begins to be broken down by soil bacteria and fungi and inorganic nitrogen nutrient ions are released (Schenck, 2003).

The highest value of plant total nitrogen content was 27.4 g kg⁻¹ by *T. a.* and the lowest 22.6 g kg⁻¹ by *T. ca.* Statistical analysis was done in order to compare tested species with the standard control (Figure 36).

Figure 36: Plants total nitrogen content just before incorporation



Means with the same letter are not significantly different at 5% (Duncan test)

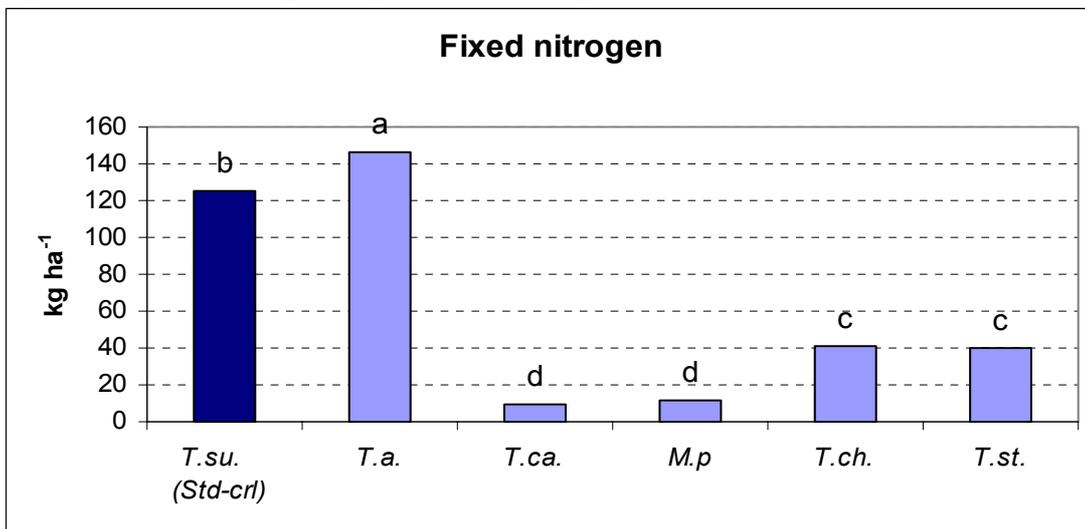
T.a., *T.st* and *M.p.* showed values that are not significantly different from the standard control, while *T.ch.* and *T.ca.* were significantly different in which the standard control showed higher value.

3.2.2.4. Biological nitrogen fixation (BNF)

Nitrogen fixed by legumes is the main nitrogen source in organic farming systems. The design of crop rotation is strongly depending on the knowledge of how much nitrogen is fixed by legumes. Also for farm scale budgeting of nitrogen, it is essential to know the amount of nitrogen fixed by the single leguminous crops (Loges *et al.*, 2000). Moreover, In order to design strategies for optimizing management of legume nitrogen fixation for maximal production with minimal nitrogen pollution of water resources, it is essential that nitrogen inputs by legumes and the subsequent fate of this nitrogen be quantitatively assessed (Unkovich and Pate, 2001). For annual legumes, the range of fixed nitrogen begins from almost nothing to 56-112 kg ha⁻¹ (Evers, 2003).

Statistical analysis was done in the period just before legumes incorporation to compare the tested species with the standard control (*T.su*) (Figure 37). Highly significant differences were obtained between *T.a.* and the standard control (*T.su.*) in which the control showed lower value. Also highly significant differences were obtained between the standard control and *M.p.*, *T.ca.*, *T.st.* and *T.ch.* in which the control showed higher value.

Figure 37: BNF in the period just before incorporation



Means with the same letter are not significantly different at 5% (Duncan test)

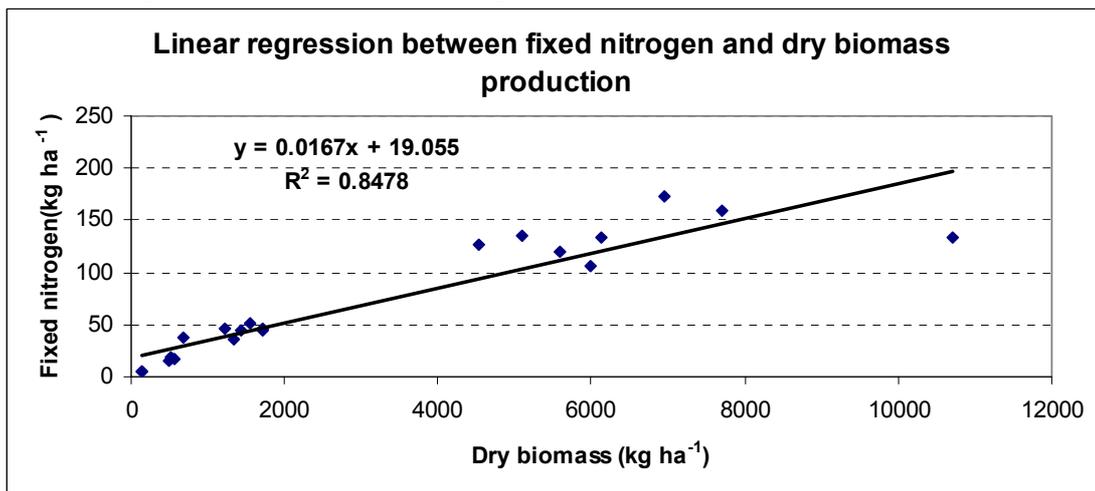
T.a. resulted in the highest amount of fixed nitrogen (146.67 kg ha⁻¹), *M.p.* and *T.ca.* resulted in the lowest amounts (11.47 and 9.91 kg ha⁻¹) while 90 and 70 kg ha⁻¹ in the previous season respectively. This is due to the high dry biomass of *M.p.* and *T.ca.* in the previous year (6 and 5 t ha⁻¹) in comparison with this year with only 0.33 and 0.32 t

ha⁻¹ respectively which resulted in a clear reduction in the amount of fixed nitrogen. This is also the case of *T.ch.* and *T.st.* which had higher values of fixed nitrogen in the previous season in comparison with this one.

Generally, legumes were less competitive during the second cropping cycle comparing to the previous one. This is in agreement with the experiment conducted by Thorup-Kristensen and Bertelsen (1996) in Denmark. This could not be only applied to nitrogen fixed, but also to all previous parameters including plants herbaceous ground cover, plants height and dry biomass production.

Linear regression was done between fixed nitrogen and dry biomass production (Figure 38) as it is an easy agronomic parameter to measure in the field to predict the amount of fixed nitrogen.

Figure 38: Linear regression between fixed nitrogen and legumes dry biomass production just before incorporation



3.3. Effect of legumes on subsequent crops

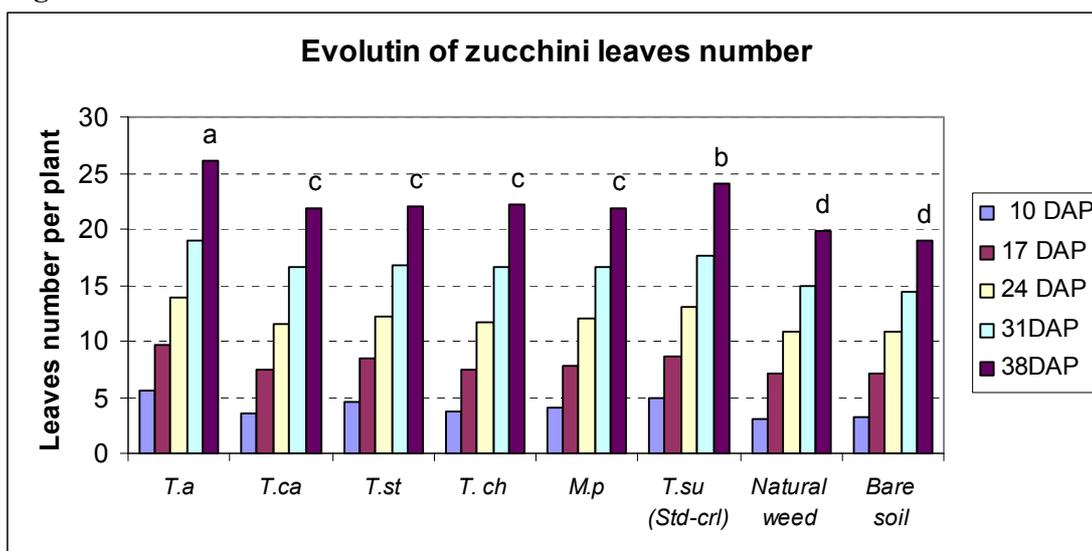
3.3.1. Impact on Zucchini growth parameters

3.3.1.1. Number of leaves

The number of leaves is an important parameter because it would affect the ability of a crop to trap solar radiation and so, manufacture carbohydrates by photosynthesis (Thwala and Ossom, 2004).

The evolution of zucchini leaves number was recorded each week for five dates starting from 10 days after transplanting (DAP). Statistical analysis done for the last observation date showed significant differences among treatments dividing them into four homogeneous groups (Figure 39).

Figure 39: Evolution of zucchini leaves number



Means with the same letter are not significantly different at 5 % (Duncan test)

As an overall observation, zucchini established after all tested species produced significantly higher number of leaves than the controls without legumes (bare soil and natural weeds) which indicated that all tested species induced positive effect on zucchini leaves number.

Similar results were obtained by Jamea (2004) in which lettuce planted following legumes showed higher number of leaves than the control in the absence of precedent legumes. Among tested species, *T. a.* achieved the highest number of leaves (26 leaves per plant) and was significantly different from the standard control in which the control showed lower value. The rest of the tested species had no significant differences among

them and were significantly different from the standard control in which the control showed higher value.

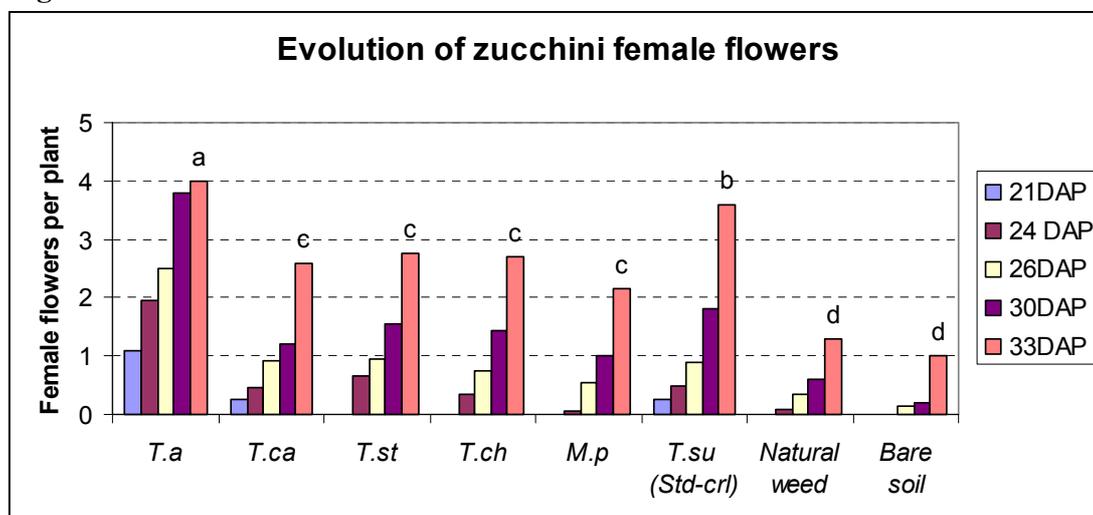
Positive correlations were found between zucchini leaves number and legumes ground cover percentage just before incorporation (61%) and legumes dry biomass incorporated in the soil (85%). In fact, green manure crops with high surface ground cover and dry biomass regulate soil surface temperatures and increase soil organic matter content thus, improve soil physical properties, control soil erosion and conserve soil moisture which provides better conditions for subsequent crop growth (Buckles *et al.*, 1998). According to this, the lowest leaves number produced by the controls without legumes incorporated in the soil (natural weed and bare soil) could be due to the absence of dry biomass effect which created poor conditions for plants growth and development, on the other hand, the absence of organic matter accumulation and nutrients release for zucchini crop.

3.3.1.2. Number of female flowers

The evolution of female flowers number during zucchini growth cycle was recorded for five dates starting from 21 DAP.

Statistical analysis (Figure 40) was done for the last observation date, four homogeneous groups were resulted).

Figure 40: Evolution of zucchini female flowers number



Means with the same letter are not significantly different at 5 % (Duncan test)

As for the number of leaves, female flowers acted the same among different treatments. Zucchini established after all tested species produced significantly higher number of female flowers compared to the controls (natural weed and bare soil) where no legumes preceded zucchini.

Among tested species, *T. a.* achieved the highest number of female flowers (4 flowers per plant) and was significantly different from the standard control in which the control

showed lower value. The rest of the tested species had no significant differences among each other and were significantly different from the standard control in which the control showed higher value.

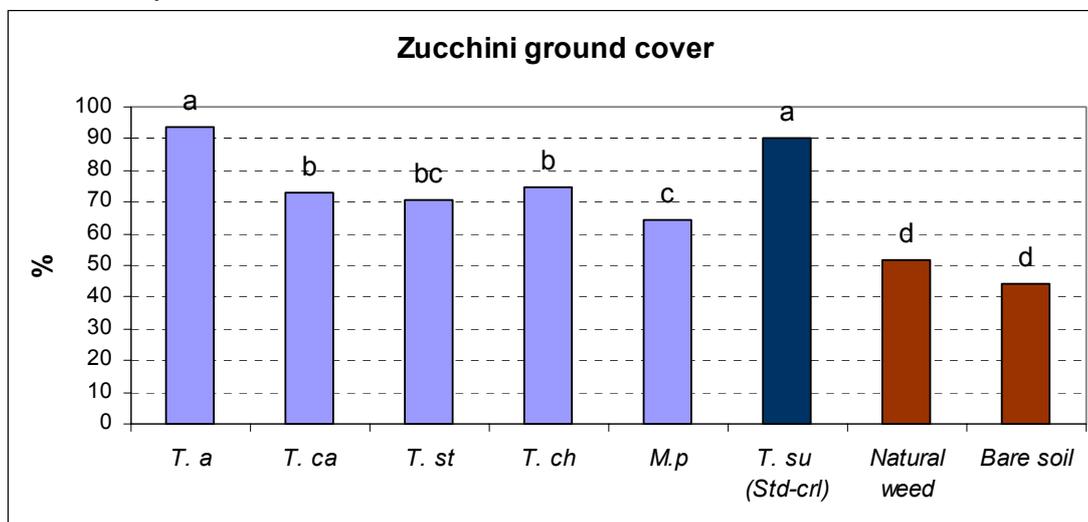
Beside to what was reported before by Buckles *et al.*, (1998) about the effect of legume dry biomass on improving soil conditions needed for subsequent crop growth, the requirement of cucurbits to high potassium and phosphorus for both good flower and fruit set and development should be considered (Lerner and Dana, 2001).

Renewed attention is being paid to green manure crops because phosphorus may become more available to crops followed certain green manure crops. It is thought that certain plants used as green manure including legumes create acidic environments around the root zone and are thus able to extract phosphorus from the soil (Entz *et al.* , 2006). Based on this, the highest flowers number in *T. a.* could be explained by having the highest dry biomass incorporated in the soil which created better acidic conditions that make more phosphorus available to zucchini plants. On the contrary, the lowest number of flowers in control treatments (bare soil and natural weed) in which no legumes were incorporated in the soil could be explained by the absence of this acidification effect that made phosphorus less available to plants. This could be confirmed by the data related to the correlation found between number of female flowers and legumes dry biomass incorporated in the soil (82%).

3.3.1.3. Soil cover

Zucchini plants with high spreading growth habit and high leaf size give it a competitive advantage over weeds and provide an opportunity as a cleaning crop to precede a less competitive vegetable in the rotation (Neeson, 2003). Statistical analysis was done for plants soil cover during the middle of the cycle in order to compare tested species with the controls (Figure 41).

Figure 41: Zucchini plants ground cover percentage in the middle of the growth cycle



Means with the same letter are not significantly different at 5 % (Duncan test).

All tested species induced a significant positive effect on zucchini ground cover percentage in comparison with controls where no legumes preceded zucchini (bare soil and weed complex). Again, thanks to green manure effect on improving soil characteristics which provides better conditions for subsequent crop growth (Buckles *et al.*, 1998).

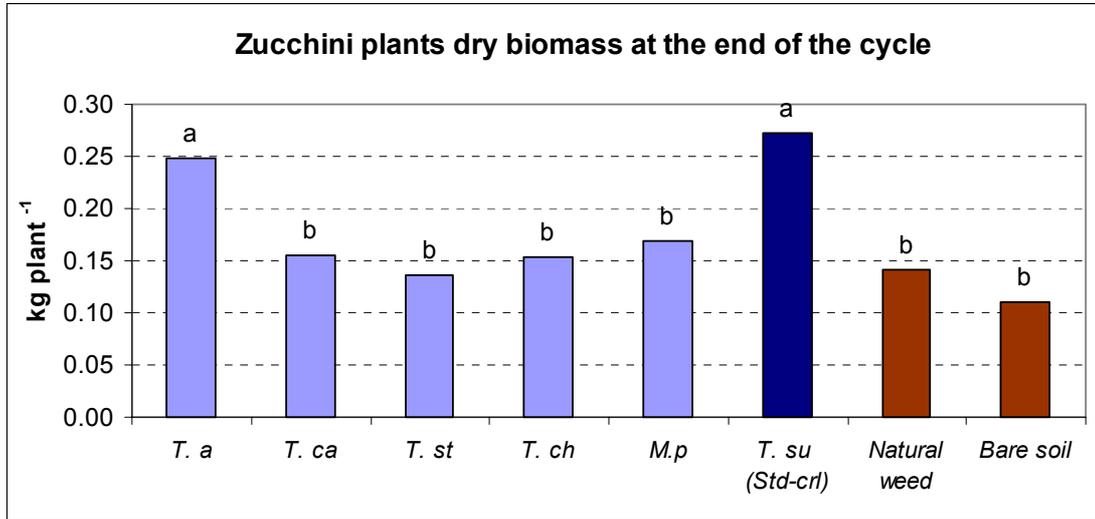
Among tested species, *T. a.* achieved the highest ground cover percentage (93.75%) which was not significantly different from the standard control (*T. su.*). The rest of the tested species were significantly different from the standard control in which they showed lower ground cover percentage.

Positive correlation of (88%) was found between zucchini cover and legumes dry biomass incorporated in the soil.

3.3.1.4. Plants dry biomass

Statistical analysis was done for zucchini plants dry biomass at the end of the cycle in order to compare tested species with the controls. Two homogenous groups were resulted (Figure 42).

Legumes seemed to have slight potential to induce positive effects on zucchini plants dry biomass compared to the controls without legumes incorporated in the soil (natural weed and bare soil). Only *T. a.* induced significant positive effect which was similar to the effect of the standard control (*T. su.*).

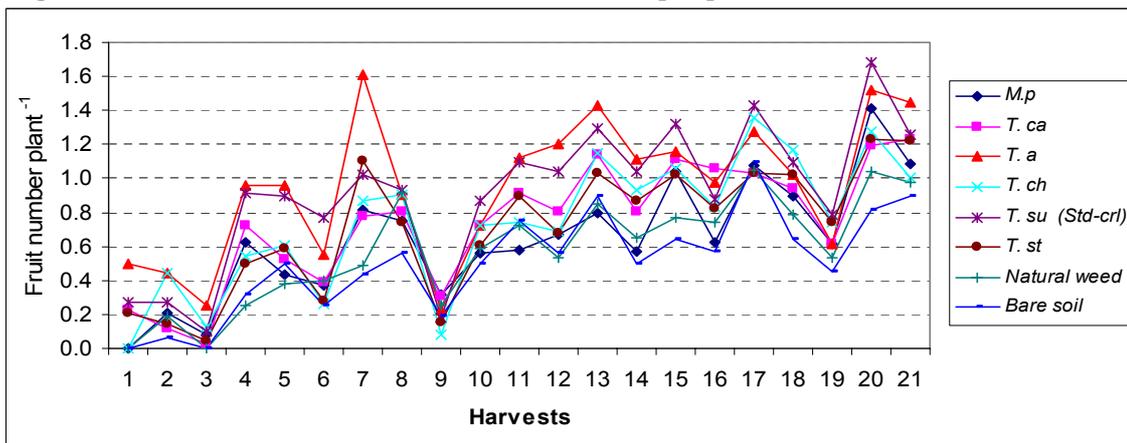
Figure 42: Zucchini plants dry biomass at the end the cycle

Means with the same letter are not significantly different at 5 % (Duncan test)

Positive correlation was found between zucchini plants dry biomass and legumes dry biomass incorporated in the soil (78%) which supported again the effect of incorporated legumes biomass on the growth and development of the subsequent crop.

3.3.1.5. Fruits number

The number of marketable zucchini fruits was recorded each harvest during the whole crop cycle. The first harvest was done 31 DAP and the last one 74 DAP. The evolution of fruits number among treatments for each harvest is presented in figure 43.

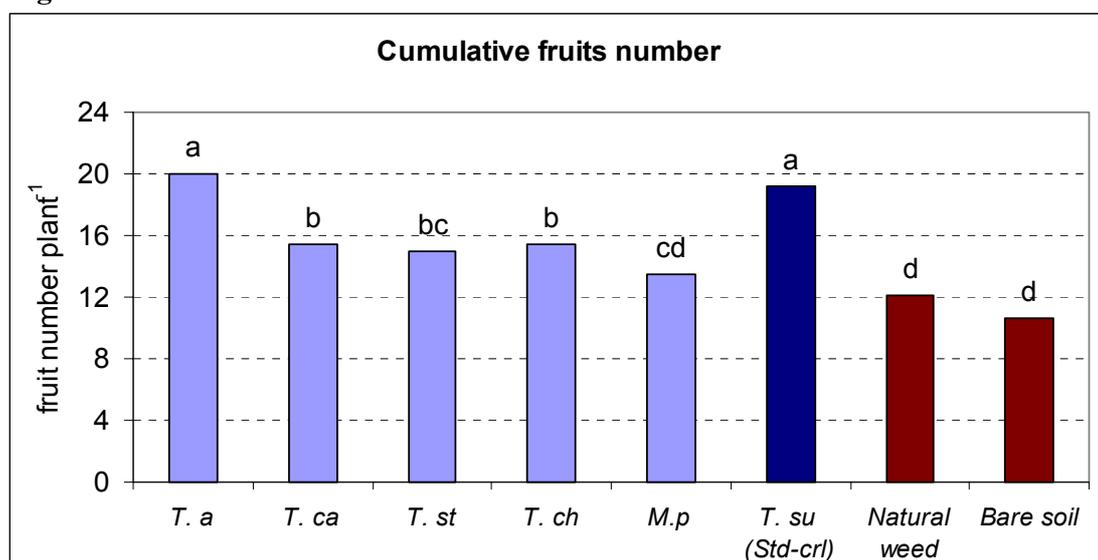
Figure 43: The evolution of zucchini fruits number per plant

Fruits number was heterogeneous throughout the season. However, the evolution shape was similar for all treatments. The maximum fruit number was recorded at the 20th harvest, then, the number started to decrease and no fruits were produced where plants reached the end of their cycle. An earlier maximum production was achieved during the 7th harvest by *T. a*.

Statistical analysis was done for the cumulative number of fruits per plant in order to compare tested species with the standard control and the controls where no legumes preceded zucchini (Figure 44).

Fruits number of all tested species, except *M. p.*, was significantly different from the controls (natural weed and bare soil) in which the controls produced lower numbers.

Figure 44: Cumulative number of zucchini fruits



Means with the same letter are not significantly different at 5 % (Duncan test)

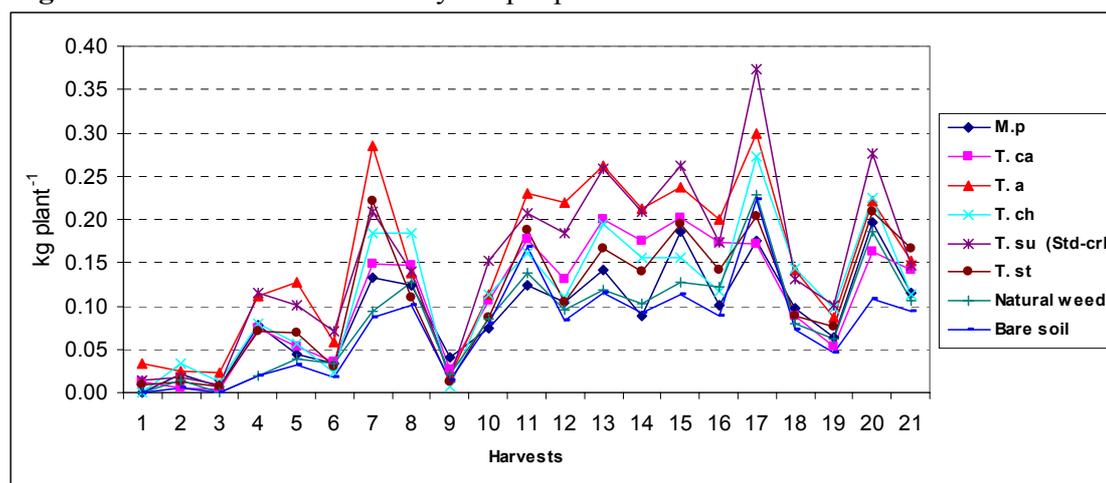
T. a resulted in the highest number of fruits (20 fruits per plant) which was not significantly different from the standard control (*T. su*). The rest of the tested species were significantly different from the standard control in which they produced lower numbers.

Positive correlation of 77% was found between number of zucchini fruits and legumes dry biomass incorporated in the soil. Additionally, positive correlations were found between number of fruits and number of zucchini leaves (89%), number of female flowers (81%) and zucchini plants ground cover percentage (89%).

3.3.1. 6. Marketable yield

The evolution of crop yield during zucchini growth cycle is presented in figure 45. As in fruits number, crop yield showed a heterogeneous trend during the crop cycle. The maximum yield was recorded in the 17th harvest, almost at the end of the season.

Figure 45: Evolution of zucchini yield per plant



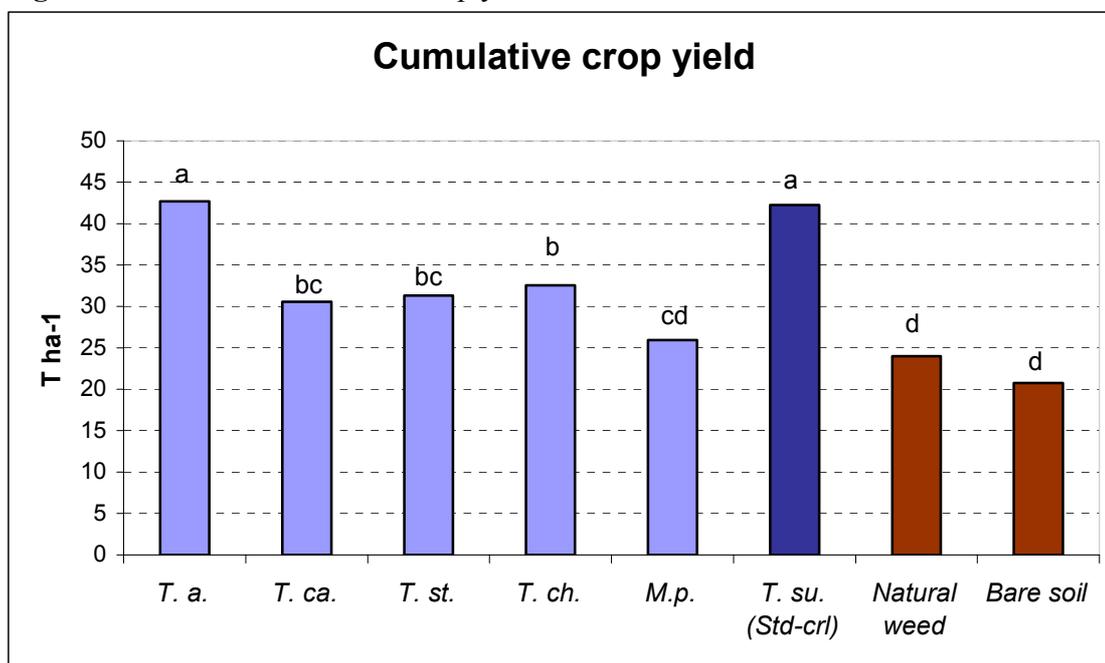
Zucchini marketable yield following tested legumes species was in average 53% higher than the controls (natural weed and bare soil) where no legumes were incorporated before planting zucchini.

Similar results were obtained by Jamea (2004) where lettuce marketable yield following legumes was in average 52% higher than following the control.

The increase of crop yield following legumes is confirmed by another research carried out by Ten Holte and Van Keulen (1989) in the Netherlands where tuber yield of sugar beet and potatoes have been increased following clovers green manure.

On another study, maize yield has been increased from 21.5% to 45.5% after legumes green manuring in comparison with the weed covered control (Caporali *et al.*, 2004) this is consistent with published reports of increased maize production following winter legumes cover crops (Blevins *et al.*, 1990; Reeves, 1994).

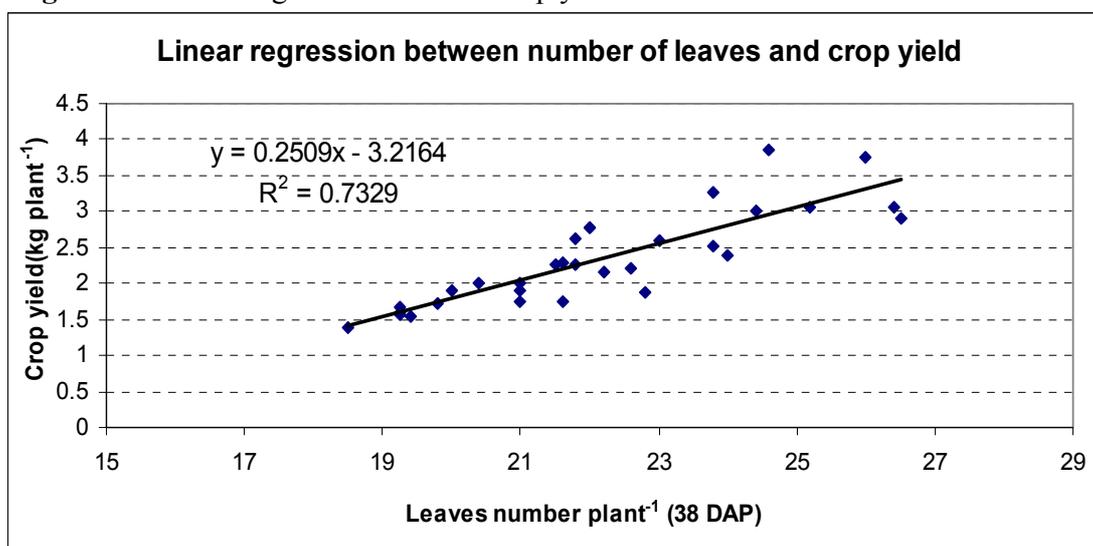
Statistical analysis was done for the cumulative crop yield (Figure 46). Zucchini yield following all tested legumes species, except *M. p.* was significantly different from the controls without legumes incorporated in the soil (bare soil and natural weed complex) in which the controls showed lower yield.

Figure 46: Zucchini cumulative crop yield

Means with the same letter are not significantly different at 5 % (Duncan test)

T. a. achieved the highest crop yield with 3.20 kg plant⁻¹ (42.66 t ha⁻¹) which was not significantly different from the standard control *T. su.* with 3.17 kg plant⁻¹ (42.26 t ha⁻¹). In fact, the yield of zucchini following *T. a.* exceeded the maximum zucchini yield recorded in Apulia region by Tesi (1990) which ranges between 20 and 40 t ha⁻¹. The rest of the tested species were significantly different from the standard control in which the control showed higher yield.

Considering that crop yield is the most relevant parameter to be studied, relationships existing between the rests of the agronomic parameters are important. Linear regression was done between zucchini crop yield and number of leaves (Figure 47) as it is easy agronomic parameters to measure in the field to predict the crop yield.

Figure 47: Linear regression between crop yield and number of leaves

3.3.1.7. Plants total nitrogen content

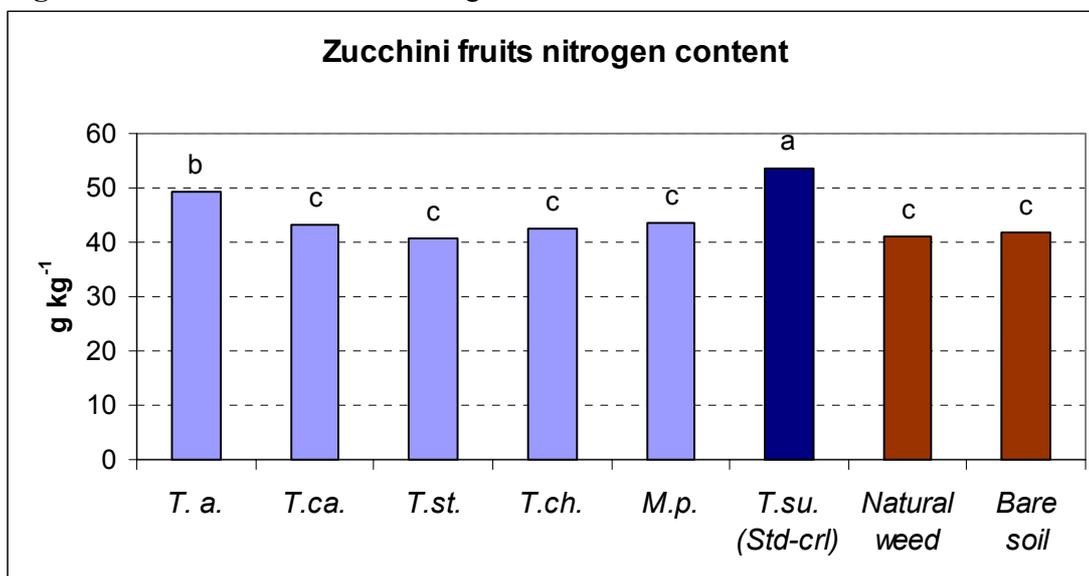
3.3.1.7.1. Total nitrogen content in fruits

Fruits nitrogen content could reflect the plant uptake of nitrogen available in the soil. Statistical analysis was done and resulted into three homogeneous groups (Figure 48).

Only *T. a.* induced positive effects on fruits nitrogen content. It showed values that were significantly higher than all tested species and the controls without legumes incorporated in the soil (bare soil and natural weed) even though its effect is still significantly less than the effect of the standard control (*T. su.*).

The rest of the tested species did not show any significant differences among each other and were also not different from the controls (bare soil and natural weed).

Positive correlation was found between zucchini fruits nitrogen content and legumes dry biomass incorporated in the soil (80%) which indicated that the dry biomass that were incorporated acted as a source for nitrogen in the fruits.

Figure 48: Zucchini fruits total nitrogen content

Means with the same letter are not significantly different at 5 % (Duncan test)

Dinesh *et al* (2000) reported that soil amended with green manures provides better conditions for crop growth. It consistently registered significantly greater microbial biomass, carbon and nitrogen flush and enzyme activities compared to unamended soil since enzyme activities are highly correlated with total biomass incorporated in the soil.

According to De Boodt *et al.*, (1961), Miller and Jastrow (1992), Tisdall (1994) and Wright and Upadhaya (1996), green manure crops may influence nitrogen accumulation in the subsequent crop in at least two ways: First, when nitrogen is available through green manure mineralization, second, green manures could increase the stability of aggregates, which can independently influenced root growth, soil aeration, soil microbiological activity and thus nutrients uptake and crop yield.

3.3.1.7.2. Total nitrogen content of aerial plants parts (leaves and stems)

Table 9 represents the total nitrogen content of aerial plants parts. The effects of all tested species and even the standard control were not significantly different from the controls where no legumes were incorporated. It seems that legumes species affected the quantitative parameters rather than the qualitative ones.

Table 11: Total nitrogen (g kg⁻¹) in Zucchini aerial parts at the end of the cycle

T. a.	T. ca.	T. st.	T. ch.	M. p.	T. su (Std-crl)	Natural weeds	Bare soil
19.1a	18.5 a	18.9 a	19.0 a	18.0 a	19.6 a	18.3 a	18.0 a

Means with the same letter are not significantly different at 5 % (Duncan test)

3.3.2. Impact on lettuce crop

One of the main constraints of organic vegetable production is lack of available N, which causes reduced yields and quality of the crops (Throup-kristensen, 2002). Lettuce crop was established as second subsequent crop in order to study the residual effect of the preceding annual self-reseeding legumes. Lettuce reflected the role of legumes in short term, since it lasted just two months.

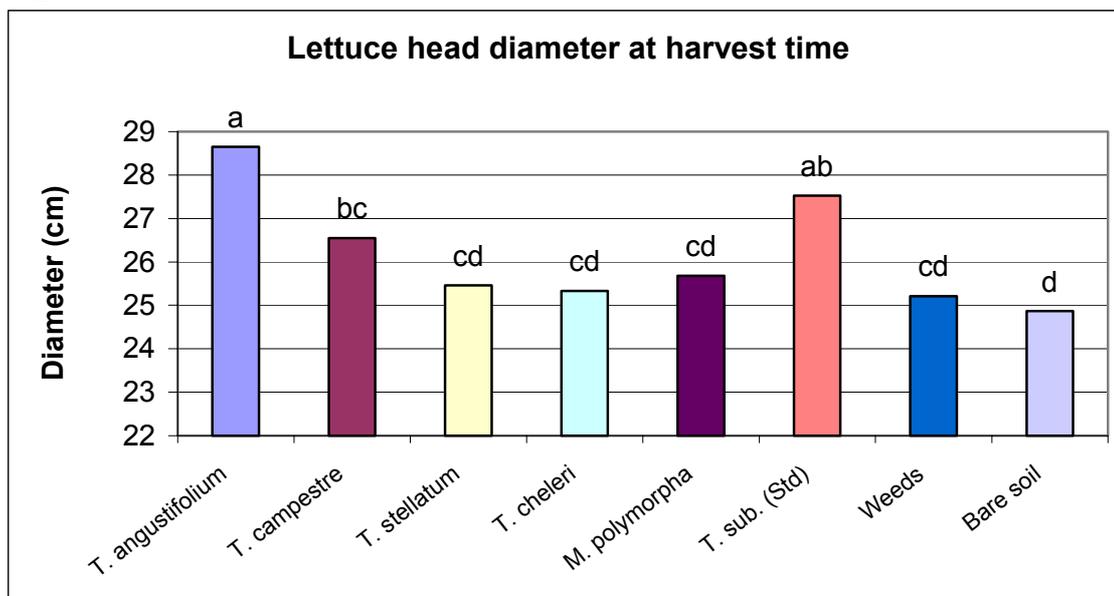
3.3.2.1. Plant head diameter

Plant head diameter is an important parameter for the assessment of lettuce growth. It is based, with plant weight, on current market standard that requires growers to pack certain number (i.e. 12 to 24) of lettuce in boxes with a certain weight (400 to 600g plant⁻¹).

Head lettuce assessment was done each two week. Results at the end of growth cycle showed that among all tested species, *T. a.* achieved the highest head diameter (28.7 cm) followed by *T. su. Std* (27.5) and *T. c.* (26.5). Instead *T. ch* , *M.p.* and controls without legume preceding (weed and bare soil) respectively achieved the lowest value (25.5, 25.3, 25.5 and 24.9)

Statistical analysis was done at harvest time in order to compare tested species with controls (Figures 49). All treatments induced a significant positive effect on lettuce head diameter in comparison with control (bare soil) where no legumes preceded lettuce.

Figure 49: Lettuce head diameter at harvest time



Means with the same letter are not significantly different at 5 % (Duncan test)

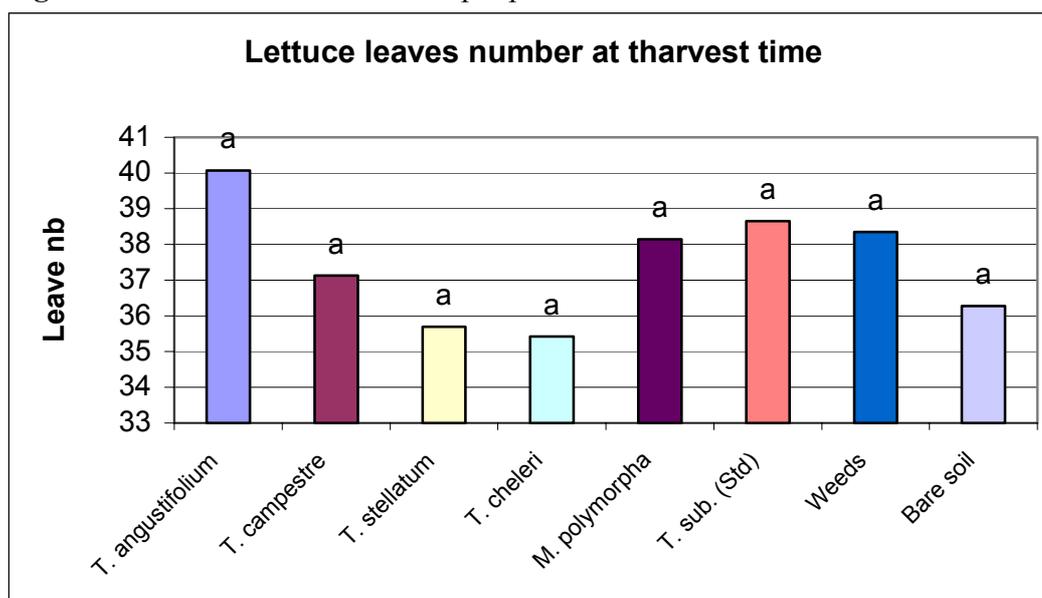
3.3.2.2. Leaves number

Leaves number assessment was done each two week to monitor lettuce growth rate. It doesn't have any effect on plant quality. However, it could indicate differences on the quantity of obtained yields.

Leaves number of lettuce at harvest time are presented in figures 50. These figure showed that lettuce established after tested annual self-reseeding legumes produced quite the same leaves than lettuce planted without any previous legume (control).

Statistical analysis done at last date of observation showed that there is no significant difference among tested species effect leaves number and in comparison with the control (absence of legumes precedent). Therefore, *T.a.* resulted in the highest leaves number (40 leaves plant⁻¹) followed by *T. su.* (39 leaves plant⁻¹) and *M. p.* (38 leaves plant⁻¹). Instead *T st.* and *T. ch.* showed the lowest value: 36 and 35 leaves plant⁻¹ respectively.

Figure 50: Lettuce Leaves number per plant at harvest time

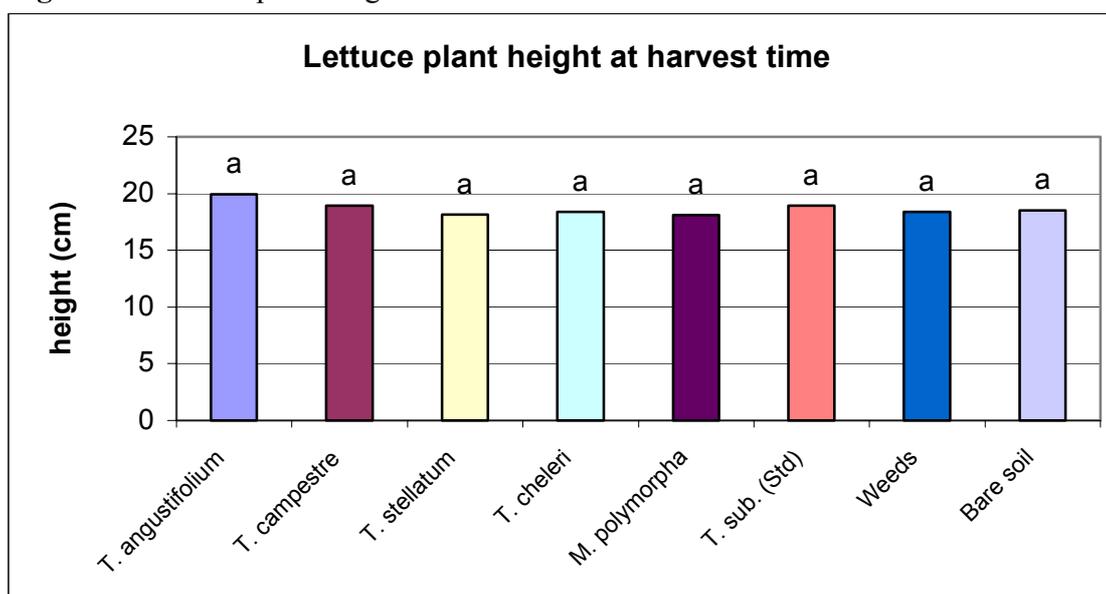


Means with the same letter are not significantly different at 5 % (Duncan test)

3.3.2.3. Plant height

The plant height of lettuce plant was measured each two week, as indicator of plant growth. No statistical differences among treatments (Figure 51) were observed. In fact, all cultivars had positive effect on the height lettuce. From agronomic point view, lettuce harvest time must be considered. For this reason we can't conclude that legumes showing positive effect on lettuce effect height can anticipate harvest time.

Figure 51: Lettuce plant height at harvest time

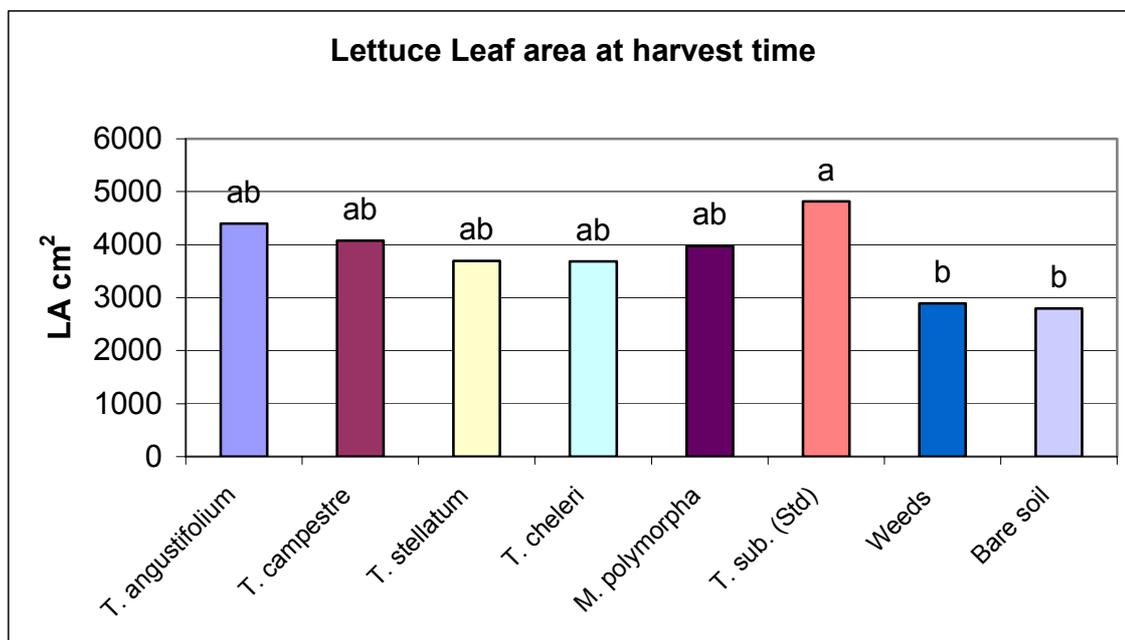


Means with the same letter are not significantly different at 5 % (Duncan test)

3.3.2.4. Leaf area (LA)

The plant leaf area (LA) was measured each two week, as indicator of plant growth. Results at the end of growth cycle showed that among all tested species, *T. a.* achieved the highest LA (4394 cm²) followed by *T. su. Sdt* (4818 cm²), *T. ca.* (4075 cm²), *T. p.* (3975 cm²) and both *T. st.* and *T. ch.* (3689 cm²). Instead Controls without legume preceding (weed and bare soil) respectively achieved the lowest values (2896 and 2799 cm²)

However no statistical differences among treatments were observed (Figure 52). Actually, all cultivars had positive effect on the height of lettuce plants.

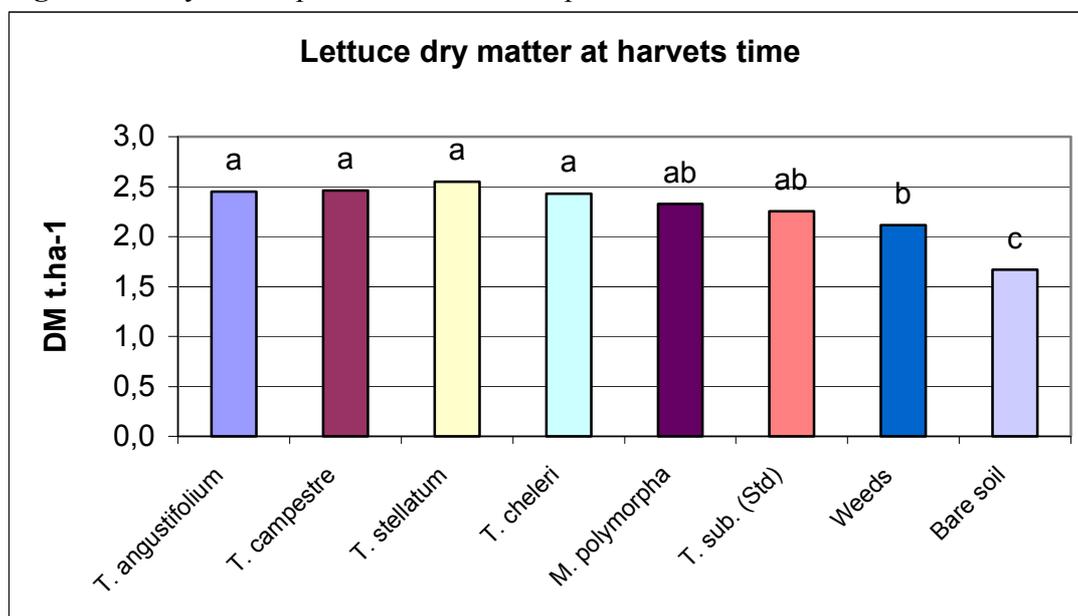
Figure 52: Leaf area (LA) at the harvest time

Means with the same letter are not significantly different at 5 % (Duncan test)

3.3.2.5. Dry matter production

The dry matter (DM) is an important parameter to assessed lettuce marketable quality. Results at the end of growth cycle showed that among all tested species, *T. st.* achieved slightly the highest DM (2.6 t ha^{-1}) followed by *T.a* and *T. ca.* (each 2.5 t ha^{-1}), *T. ch.* (2.4 t ha^{-1}) and both *T. p.* and *T. su.. Sdt* (each 2.3 t ha^{-1}). Instead Controls without legume preceding (weed and bare soil) respectively achieved the lowest values (2.1 and 1.7 t ha^{-1}).

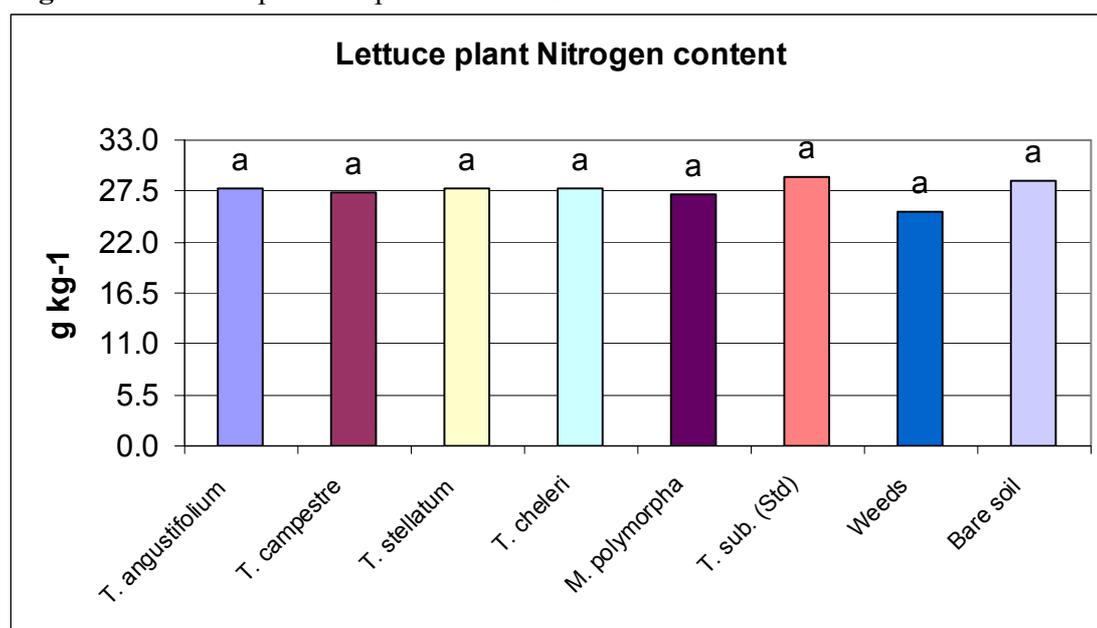
Statistical analysis at the end of growth cycle (Figure 53) showed that all species had positive effect on the lettuce production of dry matter in comparison with controls (without legumes preceding).

Figure 53: Dry matter production of lettuce plant at harvest

Means with the same letter are not significantly different at 5 % (Duncan test)

3.3.2.6. Lettuce plant nitrogen content

Statistical analysis (Figure 54) showed that all treatments had no significant difference in comparison with control on lettuce N uptake.

Figure 54: Lettuce plant N uptake at harvest time

Means with the same letter are not significantly different at 5 % (Duncan test)

3.3.2.7. Marketable yield

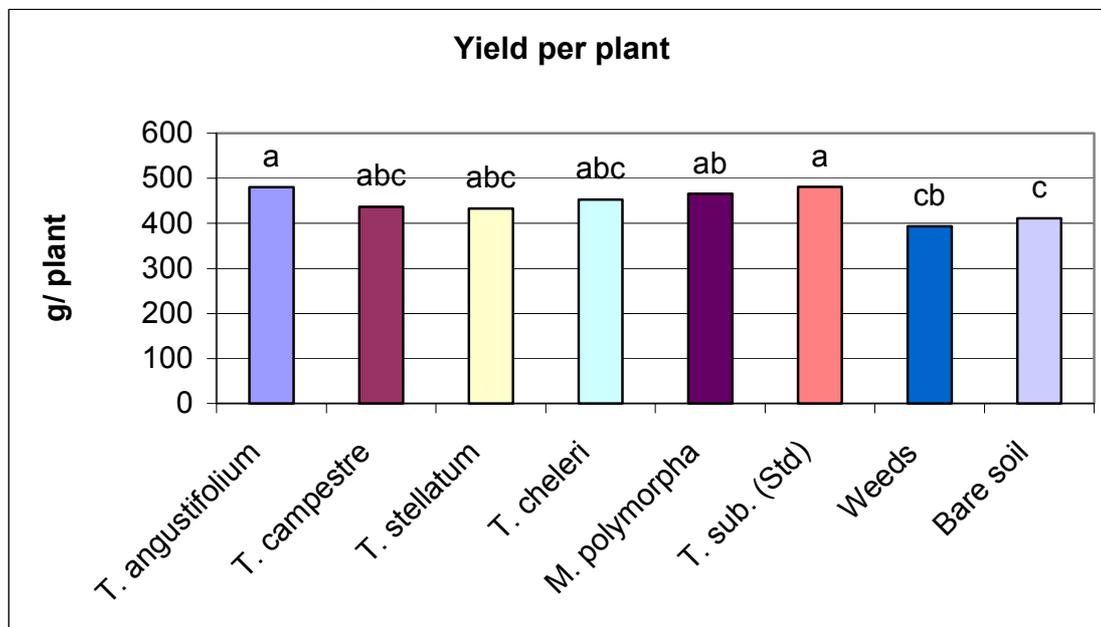
All legumes species showed a positive effect on lettuce yield compared to the control (Figure 55). In average the yield in all treatments was 24% higher than in the controls. This is in agreement with results obtained by Henry (1995) and Green *et al.* (2000). In these experiments the subsequent crops were cereals.

In fact, results at the end of growth cycle (Figure 56) showed that among all tested species, *T. su.* Std and *T. a.* achieved the highest yields (48.1 and 48 t ha⁻¹) followed by *T. p.* (46.6 t ha⁻¹). Instead controls without legume preceding (weed and bare soil) respectively achieved the lowest values (39.4 and 41 t ha⁻¹).

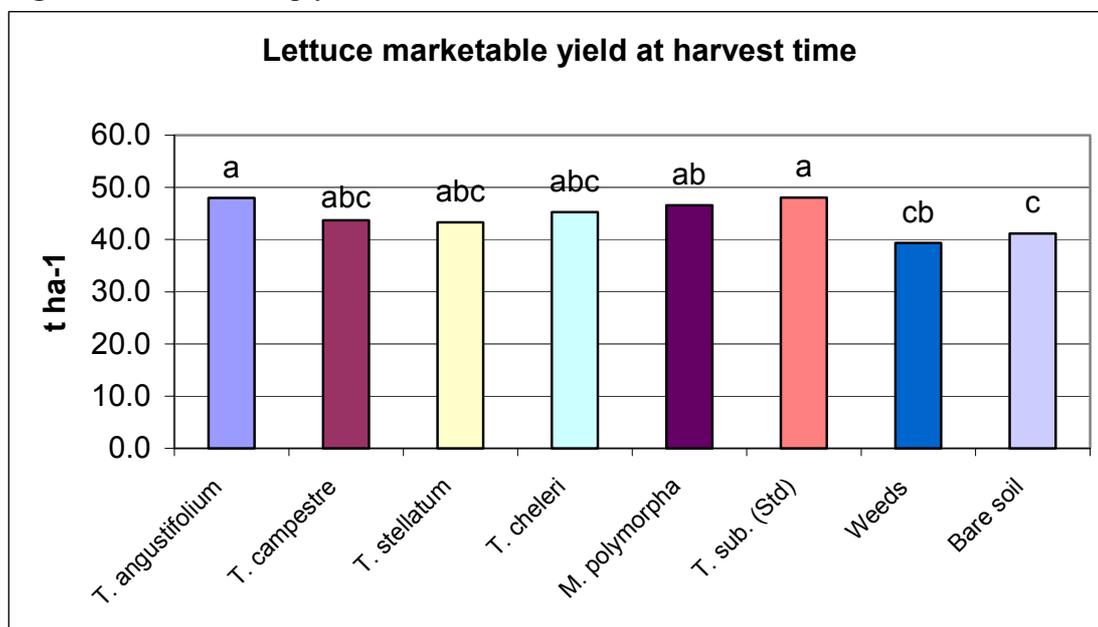
Statistical analysis (Figure 55 and 56) showed that marketable yield was increased significantly, compared to control, when legumes were grown previously to lettuce.

The increase of crop yield following legumes is confirmed by other experiments carried out by Faris *et al.*, (1986), Ten Holte & Van Keulen (1989), Wallgren & Lindén (1994), Breland (1996), Schroder *et al.*, (1997) and Thorup-kristensen (1999, 2002) . In all these experiments, the legume crops were incorporated into the soil in autumn and the subsequent crops were: maize, sugar beets, potatoes leek, carrot, onion, cabbage, green peas lettuce and cereals.

Figure 55: Lettuce yield per plant



Means with the same letter are not significantly different at 5 % (Duncan test)

Figure 56: Lettuce crop yield at harvest time

Means with the same letter are not significantly different at 5 % (Duncan test)

In addition, in a experiment carried out in Poland although with different experimental conditions, Ceglarek *et al.* (2004) reported that the best yields were obtained of celeries cultivated after green manuring of legumes (faba bean and vetch).

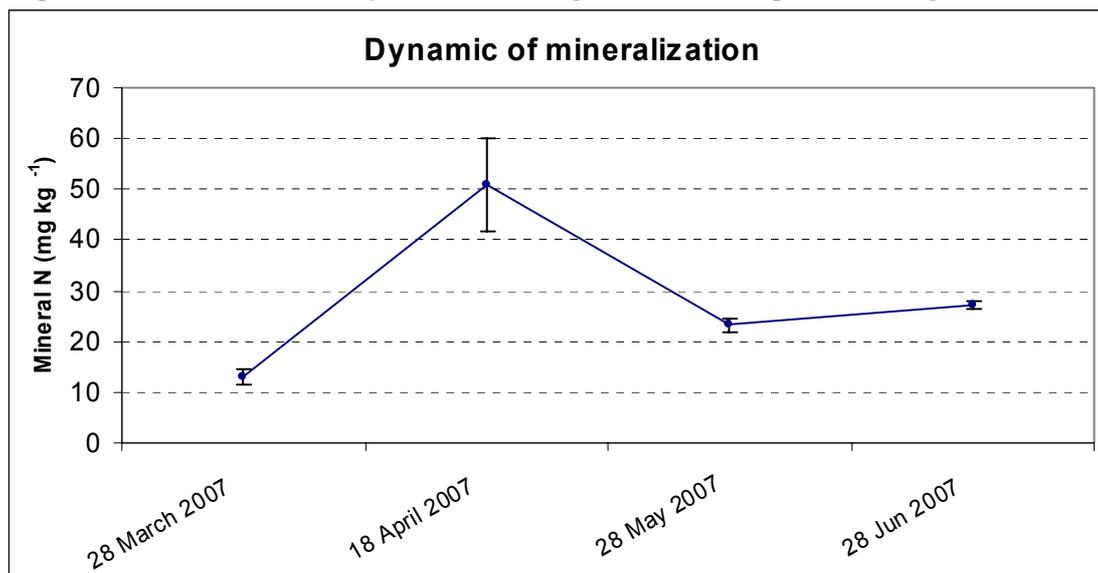
Based on the results obtained on the effect of preceding legumes on zucchini and lettuce performance, many questions concerning nitrogen dynamics in cropping systems with N₂-fixing legume crops remain to be answered. According to Aronsson (2000), after incorporation into soil, some of the legumes nitrogen may be mineralized more or less immediately and some will enter the more stable fractions of soil organic matter. More knowledge is needed about how to achieve a satisfactory degree of synchronization between the release of nitrogen from legumes material and the nitrogen demand of subsequent crops.

Many efforts have been made to quantify and to describe the temporal distribution of nitrogen mineralization after incorporation of plant material. For natural reasons, the results differ widely. Climatic conditions during autumn and winter affect microbial activity in soil. Although several studies showed that nitrogen mineralization increases after incorporation of catch crops, the effect on the yield of a following crop has been reported as slightly negative, none or positive (Aronsson, 2000)

3.4. Effect of legumes on soil available nitrogen

Figure 57 shows the dynamic of mineralization for all tested species (in average) during time in: March (six days before incorporation), April (two weeks after incorporation), May (during the middle of zucchini crop cycle) and June (at the end of zucchini crop cycle).

Figure 57 : Mineralization dynamic of average of all tested species during time.



Bars correspond to the standard error

In all treatments, an overall increase in soil mineral nitrogen was observed 15 days after incorporation (DAI) due to the onset of mineralization process. Similar results were obtained by Mazzoncini *et al* (2004). Values started to decrease till the end of May and again, slight increase was observed following that period.

As a predictor of the most favourable time to replant a crop following cover crop incorporation, the point at which microbial population are just beginning to decline would be the time when the most NO_3^- and NH_4^+ are available in the soil (Schenck, 2003). In the present study, transplanting zucchini was done about two weeks following incorporation which was also the period that showed a high increase in soil available nitrogen in all tested species. In a project performed by Schenck (2003), the most favourable replant time also appeared to be between two to three weeks after cover crop incorporation in which soil mineral nitrogen was highly available.

Statistical analysis was done for each critical date (Table 12) in order to compare tested species with the standard control (*T. su*). Mineral nitrogen content could be influenced by the release of nitrogen from soil organic matter when plant residues were left on the soil surface at the end of the last season, biological nitrogen fixation when legumes were

existed during the period October until March, nitrogen release from incorporated legumes plants throughout the period April-June and finally, zucchini plants uptake of nitrogen.

Table 12: Soil mineral nitrogen content prior to and following legumes incorporation (mg kg⁻¹).

Treatment	Date			
	28.03.07	18.04.07	28.05.07	28.06.07
	Days from incorporation			
	-6	15	55	85
<i>M. p.</i>	12.45 b	41.40 c	20.57 bc	27.15 a
<i>T. ca.</i>	20.30 a	79.40 a	25.26 ab	27.23 a
<i>T. a.</i>	9.28 b	66.25 b	24.5 ab	25.10 a
<i>T. ch.</i>	11.57 b	27.30 d	22.25 bc	27.82 a
<i>T. st.</i>	14.42 b	25.42 d	19.07 c	30.52 a
<i>T. su.</i> (Std-control)	10.37 b	65.27 b	27.85 a	25.60 a

In each column, means with the same letter are not significantly different at 5 % (Duncan test).

Statistical analysis showed significant differences between tested species 15 DAI. Although *T. ca.* resulted in the lowest dry biomass incorporated in the soil (0.32 t ha⁻¹), soil mineral nitrogen following incorporation of *T. ca.* was higher than following all tested species and even than the standard control. *T. ch.* and *T. st.* achieved the lowest values. This behaviour of *T. ca.* could be attributed to previous year added organic matter (about 5.8 t ha⁻¹) that released its nitrogen, leading to an increase in soil available nitrogen. Similar results were obtained by Jamea (2004) who reported that even legume species that did not regenerate or regenerated poorly showed a significant increase of soil available nitrogen after their incorporation because they produced high amounts of dry biomass during the previous season.

This could also be applied to explain same situation in *M. p.* that resulted in 0.33 t ha⁻¹ while about 6 t ha⁻¹ in the last year. In fact both *T. ca.* and *M. p.* resulted in the second highest dry biomass following *T. a.* at the end of the crop cycle last year so, it is important to underline that organic matter added during last season is believed to be decomposed during this year which would enrich the soil by the slow release of nitrogen in the long term.

In the third sampling date (55 DAI) values of both *T. a.* and *T. ca.* showed values that did not significantly differ from the standard control.

In the fourth sampling date (85 DAI), no significant differences were obtained among all tested species and the standard control though an increase in all values except the standard control (*T. su.*) was observed when compared with the previous date (55 DAI), in addition to a clear increase in values of both *T. ch.* and *T. st.* in comparison with others in the same date (85 DAI) and in comparison with their values in the two previous dates (15 and 55 DAI).

In all tested species (except *T. ch.* and *T. st.*), soil mineral nitrogen content consequent to mineralization peaked 15 DAI. *T. ch.* and *T. st.* it peaked 85 DAI even though their peak values were not significantly different from the values of the rest of species at the same date.

Differences in mineralization peak periods are likely due to differential aging of residues at the time of incorporation (Schenck, 2003). The quality of plant residues added to soil determines both the rate of decomposition and the dynamic of mineral nitrogen (Mary *et al.*, 1996; Kara, 2000). C/N ratio plays a critical role in nitrogen budget in soil during decomposition of added organic material since higher available carbon content in the added material was found to be leading the nitrogen-immobilization during the early phase of decomposition (Reinertsen *et al.*, 1984). In other words, the amount of nitrogen mineralized or immobilized appeared to be dependent on the type of the plant material, particularly its C/N ratio (Fog, 1988). The kinetics of decomposition are much more related to the biochemical composition of the residues, such as soluble carbon, cellulose and lignin content (Reinertsen, 1984; Kirchmann and Bergquist, 1989).

So, in addition to the fact that *T. ch.* and *T. st.* produced dry biomass of only 1.36 and 1.19 t ha⁻¹ at the end of this season and 3.5 and 3.4 t ha⁻¹ at the end of last season respectively which were lower than all tested species, the explanation of the lower soil mineral nitrogen values in both *T. ch.* and *T. st.* 15 and 55 DAI in comparison with other species could be their nature supposing that they could have higher C/N ratio which makes lower nitrogen available from them.

On the other hand, the peak time for those two species was delayed from the other species and although still low, an increase in values could be expected but can not be tested since no more nitrogen analysis was done following that period (85 DAI). The reason of this kind of delay could be the composition of *T. ch.* and *T. st.*, supposing that their nitrogen compounds were initially more resistant to microbial decomposition action which took more time for nitrogen to be released latter (85 DAI). Similar results and attributions were obtained by (Schenck, 2003) who stated that this pattern of release from leguminous green manure is specially beneficial for some subsequent crops if older plants require much greater nitrogen inputs than the seedlings plants.

Zucchini was transplanted in 20.04.07 (17 DAI) and started to uptake available soil nitrogen starting from that period. So, the subsequent crop consumption could explain the reduction in available nitrogen values 55 DAI.

Amato *et al.*, (1987) stated that where leguminous residues are ploughed in, extensive net N mineralization often occurs within 3-4 months of incorporation. However, the amount of N actually contributed by green-manure crops to subsequent crops varies considerably and is dependant on environmental conditions, Carbon (C) to N ratios (C:N) of the crop residues, soil available N concentration, and soil microbial activity. Therefore, it is important to assess how much N the green-manure crop provides and whether the time course of release of inorganic N from the decomposing residues coincides with the subsequent crop N demand (Shennan, 1992).

Schenck (2003) reported that as the plant material becomes available for microbial colonization, the microbial populations increase and then slowly diminish again as the nutrients are consumed. Likewise, the released nitrogen compounds increase and then decrease as they are either taken up by weeds, another plant crop or immobilized into other compounds by microorganisms. Another explanation of nitrogen decrease could be leaching consequent to rainfall events during that period (Figure 36).

The following increase in soil available nitrogen 85 DAI and which might continue to increase after that period in species that already reached their peaks 15 DAI could be explained by the existence of two microbial population peaks. The suggested explanation was that the first population increase may represent microorganisms that are consuming and multiplying on readily available organic compounds, carbohydrates and amino acids. The second peak could be composed of other microbial species that decompose more resistant compounds (Schenck, 2003). Similar results were obtained by Hue and Silva (2000) who observed that ammonium released from a legume green manure was highest between two to four weeks after incorporation and remained low between six to ten weeks. After ten weeks, the ammonium in soil began to increase again as long as 16 weeks. In the case of the present study, 85 DAI in which mineral nitrogen started to increase represents the week number 12 after incorporation, so more increase in soil available nitrogen during time following that period could be highly expected.

3.5. Overall evaluation of the tested species

Table 13: Ranking of evaluated species according to the self-establishment capacity and impact on subsequent crops

Rank		2 nd legumes growth cycle				Impact on subsequent crops								Sum/60
		Legumes performance				Zucchini				Lettuce				
		Plant height cm	Crop ground cover %	Dry matter DM t ha ⁻¹	BNF kg ha ⁻¹	Leaves number	Cover crop %	DM t ha ⁻¹	Yield t ha ⁻¹	Head diameter cm	Height cm	LA cm ²	Yield t ha ⁻¹	
1	<i>T. angustifolium</i>	5	5	5	5	5	5	5	5	5	5	5	5	60
2	<i>T. subteraneum</i> (Cr1 1)	5	5	4	4	4	5	5	5	4.5	5	5	5	56.5
3	<i>T. cheleri</i>	4	4	3	2	3	4	4	4	2.5	5	4.5	4	44
4	<i>T. campestre</i>	4	3	3	2	3	4	4	3.5	2.5	5	4.5	4	42.5
5	<i>T. stellatum</i>	4	4	3	3	3	3.5	4	3.5	2.5	5	4.5	4	43.5
6	<i>M. polymorpha</i>	4	3	0	1	3	3	4	2.5	2.5	5	4.5	4.5	37.5
7	<i>M. rigidula</i>	0	0	0	0	0	0	0	0	0	0	0	0	0
8	<i>M. radiata</i>	0	0	0	0	0	0	0	0	0	0	0	0	0

5 corresponds to the maximum value and 1 to the lowest

In terms of agronomic self-establishment capacity and positive effect on subsequent crops, *T.* species were on average more and better adapted to the pedo-climatic conditions of the experimental site.

Regarding the Trifolium spp., *T. angustifolium* showed to be a very promising one compared to all the other species, in terms of high capacity in self-establishment and positive effects on all growth parameters of subsequent species

For Medicago species, *M. polymorpha* regenerated poorly instead *M. radiata* and *M. rigidula* did not regenerate. It can be concluded that they were not suitable to the pedo-climatic conditions of the experimental site.

General conclusions and recommendations

According to the results obtained during the first cropping cycle, tested species belonging to the genus *Trifolium* appeared to be generally more adapted to the pedo-climatic conditions of the studied area than the genus *Medicago*.

Over the seven tested species (four *Trifolium* and three *Medicago*), five were suitable to the site's conditions and the two species considered as not suitable were *Medicago radiata* and *Medicago rigidula*.

Moreover, between all the tested species, including *Trifolium subterraneum* cv. ANTAS (Cr11), *Trifolium angustifolium* and *Medicago polymorpha* were the most performing and promising ones regarding most of the tested agronomic parameters.

The integration of the tested species into Mediterranean cropping systems should be based on the different performances exhibited by each species. In this context, *Trifolium angustifolium* and *Medicago polymorpha* may be appreciated for their high nitrogen fixation, rapid soil covering and abundant flowering. Consequently, they may play an important role in the management of soil fertility and can be a key-element in enhancing biodiversity of orchard.

Trifolium angustifolium, *Trifolium cherleri*, *Trifolium stellatum* and *Medicago polymorpha* they showed good seed production, they should be able to germinate without any human intervention for sowing and they should have the ability to control weeds, making them very interesting since weed control is a major problem under organic farming systems and to break pest and diseases cycles.

Besides, *Trifolium angustifolium*, *Trifolium campestre* and *Medicago polymorpha* yielded large amounts of dry matter which is an asset since they are suitable to be used as green manure, for hay, silage and as highly acceptable forage to livestock whether grazed or fed. Further investigations should concern these last aspects. Consequently, their cultivation would be a large benefit especially for mixed organic farming system.

Medicago polymorpha, *Trifolium angustifolium* and *Trifolium subterraneum* could be used as intercrop for orchard for example as they have the highest values in plant height (more than 40 cm) so they can cover soil and help in managing weeds as for the other species could be used in association with other crops that have higher plant height or as cover crop.

Trifolium angustifolium, *Trifolium stellatum* and *Medicago rigidula* could be used in areas where dry seasons arrive early, so these species could escape dry seasons regarding flowering stage.

While in second cropping cycle and based on the results obtained, preliminary conclusions can be set up for the integration of annual self-reseeding legumes into Mediterranean organic cropping systems.

As an overall observation, tested species seemed to be less performing during this cropping cycle compared to the first one. This is in consistent with results obtained by (Jamea, 2004). Consequently, it might be concluded that a sown annual self-reseeding legumes grow quite better than self-established one.

Over the seven tested species (four *Trifolium* and three *Medicago*), the ones belonging to the genus *Trifolium* appeared to be more adapted to the pedo-climatic conditions of the studied area in their second cropping cycle and to have better effects on the subsequent crop than the genus *Medicago*. In fact, both *Medicago radiata* and *Medicago rigidula* did not regenerate while *Medicago polymorpha* regenerated very poorly and had no effect on zucchini (first subsequent crop) yield; so, it could be concluded that they are not appropriate to self-reseed and to be used as green manure under Apulia conditions.

Well regenerated annual self-reseeding legumes showed to have induced statistically positive effects on different zucchini quantitative and qualitative parameters. Zucchini crop yield was increased by 53% (in average) compared to control when no legumes preceded zucchini. Therefore, it is of great importance to underline the consistent contribution of legumes in improving soil fertility. In fact, regenerated species appeared to sustain optimum level of soil available nitrogen needed by zucchini, therefore, their integration into organic cropping systems in the Mediterranean region could provide reasonable alternatives for farmers to manage nitrogen supply and to enhance long-term soil fertility which will minimize the reliance on off-farm inputs.

Taking into consideration that self establishment in the field is also evaluated through legumes dry biomass production for the purpose of green manuring, *Trifolium angustifolium* was the best performing species since it accumulated the highest amount of dry biomass and resulted in the highest quantity of fixed nitrogen. In addition to that, zucchini and Lettuce yield was the highest when *Trifolium angustifolium* preceded and it was the only one that had a positive effect on fruit nitrogen content. Therefore, this species is highly recommended to be integrated into vegetable rotation programme within Mediterranean region.

Moreover, because of its high dry biomass production (8.22 t ha⁻¹ in average for the two growing cycle) *Trifolium angustifolium* could be considered to be suitable for hay and silage production and as highly acceptable forage to livestock, whether grazed or fed, because of their high nitrogen content and thus feeding value. Consequently, their cultivation would be of large benefit especially for mixed organic farming systems.

The rest of *Trifolium* species appeared to induce a significant positive effect on zucchini and lettuce yield compared to control even though of lesser effect than *Trifolium angustifolium*. In fact, the dry biomass they produced was not significantly different from *Medicago polymorpha* which had no effect on zucchini yield. Therefore, it could

be concluded that the residual effect of the dry biomass produced by *Trifolium* spp. left on the soil surface at the end of the last season acted in a certain way that synchronized better with zucchini and lettuce crop requirements than *Medicago polymorpha*. However, further investigations should concern this last aspect.

In fact, the legumes precrop effects on other subsequent crops should be investigated. Zucchini and lettuce were grown after legumes incorporation and lasted just for four months. Such period does not demonstrate the real role of legumes in rotation programme since legumes dry biomass could have long term effect on the next subsequent crops. This is in agreement with results obtained by (Wehbe, 2005) in which crop yield of the third subsequent crop (wheat) as affected by legumes incorporation was higher than the first and the second ones. For this reason, following crops in this investigation programme will accomplish the obtained results and the conclusion can be drawn more clearly.

However, this experiment should be continued and pursued for several years to allow drawing definitive conclusions concerning each of the tested species, because the agronomic performances of some species may change during the next growth cycles.

Finally, it would be recommended to:

- Investigate the effect of cover crop of the most promising species under olive or grapevine production system.
- See the effect of these species on weeds, pests and diseases as they would attract several beneficial insects through their different colored flowers and to grow some species in mixture to increase biodiversity.
- Study the soil microbial activity to identify and to study the available bacteria and specific rhizobia for each tested species.
- Conduct this study in different sites within the Mediterranean region because some species that were less performing in Apulia might be better performing in other parts and *vice versa*,
- Enlarge the screening of other Mediterranean native species since the region has very rich biodiversity,
- Enlarge the study to investigate on the use of self-reseeding legumes species as cover crops in permanent organic crops or its use in mixed farming systems,
- Set up another research focusing on the performance of tested *Medicago* spp. in order to assess their self-establishment over several years supposing that their behaviour can be improved with time, as reported by Cocks (1993), so that those medics can be exploited for other purposes, rather than green manuring, such as sustaining pastures in mixed farms.

References

- Abd El-Moneim A.M. and Cocks P.S. (1986).** Adaptation of *Medicago rigidula* to a cereal-pasture rotation in North-West Syria. *Journal of agricultural science*, 107: 179-186.
- Al-Bitar L. (2005).** Annual self reseeding legumes, a key component of sustainability in Mediterranean organic cropping system. University of Tuscia-Viterbo-Italy. PhD thesis.
- Andres T.C. (2004).** Web site for the plant family Cucurbitaceae & home of The Cucurbit Network. <<http://www.cucurbit.org/family.html>>.
- Amato M., Ladd J.N., Ellington A., Mahoney J.E., Taylor A., and Walscott D. (1987).** Decomposition of plant material in Australian soils. IV Decomposition *in situ* of ¹⁴C and ¹⁵N-Labelled legume and wheat materials in a range of Southern Australian soil. *Australian Journal of Soil Research*, 25:95-105
- Aronsson Helena, 2000.** Nitrogen turnover and leaching in cropping systems with ryegrass catch crops. PhD thesis, Swedish University of Agricultural Sciences-Department of Science Sciences-Uppsala 2000.
- Ashraf M., Mahmood T., Azam F. and Qureshi R.M. (2004).** Comparative effects of applying leguminous and non-leguminous green manures and inorganic N on biomass yield and nitrogen uptake in flooded rice (*Oryza sativa* L.). *Biology and fertility of soils*, 40:147-152.
- Ayanaba A. and Lawson T. L. (1977).** Diurnal changes in acetylene reduction in field-grown cowpeas and soybeans. *Soil biology and biochemistry*, 9: 125-129.
- Bàrberi P., Caporali F., Campiglia E. & Mancinelli R. (1998).** Weed community composition in a mixed farming system in Central Italy. *Proceedings of a Workshop on Mixed Farming Systems in Europe*, Dronen (NL), 25-28 May, ed. H. Van Keulen, E.A. Lantinga e H.H. Van Laar, A.P. Minderhoudhoeve-reeks nr. 2, Landbouwniversiteit Wageningen, 71-77 p.
- Batte M.T., Forster, D.L., Hitzhusen, F.J. (1993).** Organic agriculture in Ohio: an economic perspective. *J. Prod. Agric.* 6, 536–542.
- Beardsell D. (1995).** Nutrient deficiency symptoms of plants. *Agriculture notes*, April. <<http://www.dpi.vic.gov.au/DPI/nreninf.nsf/Home+Page/DPI+InfoSeries~Home+Page?open>>.
- Becker M., Ladha J. K. and Ali M. (1995).** Green manure technology: potential, usage and limitations. A case study for lowland rice. *Plant and soil*, 174(1-2): 181-194.
- Bell F. and Nutman P. S. (1971). Experiment on nitrogen fixation by nodulated .
- Bell F. and Nutman P. S. (1971).** Experiment on nitrogen fixation by nodulated Lucerne. *Plant and Soil*, Special Volume. 231-204 p.
- Bergersen F.J. and Turner G.L. (1983).** An evaluation of ¹⁵N methods for estimating nitrogen fixation in a subterranean clover-perennial ryegrass sward. *Australian journal for agricultural research*, 34: 391-401.
- Bianco V. (1990).** Asteraceae. Lattuga (*Latuca staiva* L.) in: Bianco and Pampini (coords.). *Orticoltura. Patron*, Bologna, pp. 270-319.

*: date of the last access to the web sites: December 20th, 2007

- Black C.A., Evans D.D., White J.I., Ensminger L.E. and Clarck F.E. (1982).** Methods of soil analysis. In: Page A.L. (ed.). Part 2. Chemical and microbiological properties. 2nd ed. American Society Agronomy publisher, Madison, Wisconsin USA.
- Blevins R.L., Herbek J.H. and Frye W.W. (1990).** Legume cover crops as nitrogen source for no-till corn and grain sorghum. *Agronomy journal*, 82: 769-772.
- Blondel J. and Aronson J. (1999).** Biology and wildlife in the Mediterranean region. Oxford University Press, Oxford, UK.
<<http://www.oup.com/us/catalog/general/subject/LifeSciences/Ecology/?ci=0198500351&view=usa>>.
- Bond, W., Moore, H.C., Atkinson, R.J., Bevan, J.R., Lennartsson, M.E.K. (1998).** Changes in the weed seedbank following different weeding treatments in drilled salad onion and carrot crops grown in organic and conventional systems. *Biol. Agric. Hortic.* 16, 203–215.
- Bounjemat M. (1990).** In rapport annuel 1987 / 88, station centrale des plantes fourragères (INRA: Rabat, Morocco).
- Bounjemat M., Beale P.E. and Robson A.D. (1992).** Annual Medicago species in Morocco and their abundance. *Australian Journal Agriculture Research*. 43(3).
- Bowren K.E., Cooke D.A. and Downey R.K. (1969).** Yield of dry matter and nitrogen from tops and roots of sweet clover, alfalfa and red clover at five stages of growth. *Canadian journal of plant science*, 49: 61-68.
- Boyhan G.E., Granberry D.M. and Terry Kelley W. (1999).** Squash: Commercial vegetable production. University of Georgia.
<<http://pubs.caes.uga.edu/caespubs/pubs/PDF/C527.pdf>>.
- Bremner J.M. and Mulvaney C.S. (1982).** Nitrogen total. In: Methods of soil analysis. A.L. Page (ed.) Agron. No.9, 2nd ed., Am. Soc. Agron., Madison, WI, USA. 595-624 p.
- Brygoo Y. (2001).** “*Pseudopeziza medicaginis*(Lib.) Sacc. f. sp.*medicaginis-lupulinae*Schmied”. INRA (Institut National de la Recherche Agronomique) <<http://www.inra.fr/hyp3/pathogene/6psemed.htm>> [last date of consultation: 3 August 2006].
- Buckles, D., Triomphe, B. and Sain, G. (1998).** Cover crops in Hillside Agriculture. Farmer innovation with mucuna. IDRC/CIMMYT, Canada, pp 218
- Burdass D. (2005).** The nitrogen cycle. *Microbiology today*, 11: 188-190.
<http://www.socgenmicrobiol.org.uk/pubs/micro_today/pdf/110509.pdf>.
- Burket J., Sandeno J., Hemphill D. and Dick R. (2003).** Cover crop nitrogen for summer vegetable crops. Oregon state university.
<<http://extension.oregonstate.edu/catalog/pdf/em/em8803-e.pdf>>.
- Buys, J. (1993).** Conversion towards organic agriculture in Russia: a preliminary study. *Biol. Agric. Hortic.* 10, 125–140.
- Campiglia E. (1999).** Colture di copertura utilizzate in agroecosistemi mediterranei. *Rivista di agronomia*, 33: 90-103.
- Caporali F., Nannipieri P., Paoletti M.G., Onnis A. and Tomei P.E. (1989).** Concepts to sustain a change in farm performance evaluation. *Agricultural ecosystem and environment*, 27: 579-595.

- Caporali F. and Onnis A. (1991).** Validity of rotation as an effective agroecological principle for a sustainable agriculture. *Agriculture Ecosystem and Environment*, 41: 101-113.
- Caporali F. and Campiglia E. (2001).** Increasing sustainability in Mediterranean cropping systems with self-reseeding annual legumes. In : Gliessman S. R. (ed.). *Agroecosystem sustainability. Developing practical strategies*. CRC, Boca Raton, pp. 15-27.
- Caporali F. (2004).** *Agriculture and Health- the challenge of Organic agriculture*. EDITEAM, Ferrara, Italy.
- Caporali F., Campiglia E., Mancinelli R. and Paolini R. (2004).** Maize performances as influenced by winter cover crop green manuring. *Italian journal of agronomy*, 8(1): 37-45.
- Chalk P.M. (1985).** Estimation of N₂ fixation by isotope dilution: an appraisal of techniques involving ¹⁵N enrichment and their application. *Soil biology and biochemistry*, 17: 389-410.
- Chatterton B. and Chatterton L. (1984).** Alleviating land degradation and increasing cereal and livestock production in north Africa and the Middle East using annual *Medicago* pastures. *Agriculture, Ecosystems and the Environment*, 11:117-129.
- Chatterton L. and Chatterton B. (1996).** Sustainable Dryland Farming: Combining Farmer Innovation and Medic Pasture in a Mediterranean Climate. ISBN: Publisher: CambridgeUniversityPress1996.
- Chapman H.D. and Pratt J. (1961).** Methods for soils, plants and waters. University of California.
- Ceglarek R., Bajkowska A., and Rosa R. (2004).** The effect of green manure and soil liming on the yielding of rooted Celery, "Edward" cv <<http://www.ejpau.media.pl/volume7/issue1/horticulture/art-09.htm>>.
- Clark, M.S., Horwath, W.R., Shennan, C., Scow, K.M., Lanini, W.T., Howard, F. (1999).** Nitrogen, weeds and water as yield-limiting factors in conventional, low-input, and organic tomato systems. *Agriculture, Ecosystems and the Environment*, 73:257-270.
- Cocks P.S. and Ehrman T.A.M. (1987).** The geographic origin of frost tolerance in Syrian pasture legume. *Journal of Applied. Ecology*, 12: 678-683.
- Cocks P.S. (1993).** Seed and seedling dynamics over four consecutive years from a single seed set of six annual medics (*Medicago spp*) in north Syria. *Experimental Agriculture*,29:461-472.
- Covell S., Ellis R., Roberts E.H. and Summerfield R.J. (1986).** The influence of temperature on seed germination rate in grain legumes. *Journal of Experimental Botany*, 37: 705-715.
- Crawford R. M. M. (1989).** Studies in plant survival: ecological case histories of plant adaptation to adversity. *The Journal of Ecology*, Vol. 77, No. 4 (Dec., 1989), p. 1179
- Cuevas, G., Blazquez, R., Martinez, F., Walter, I. (2000).** Composted MSW effects on soil properties and native vegetation in a degraded semiarid shrub land. *Compost Sci. Utilization*: 8, 303-309.
- Dallman P. (1988).** Mediterranean skies above California <<http://www.mediterraneangardensociety.org/climate/pdallma1.cfm>>.

- Danso S.K.A. (1985).** Methods of estimating biological nitrogen fixation. In: Sali H. and Keya S.O. (eds). Biological nitrogen fixation in Africa. Rhizobium MIRCEN, Nairobi, Kenya, pp. 224-244.
- Danso S.K.A., Hardarson G. and Zapata F. (1985).** Assessment of dinitrogen fixation potentials of forage legumes with ^{15}N technique. Potentials of forage legumes in farming system of sub-Saharan Africa-proceeding of a workshop held at ILCA, Addis Ababa, Ethiopia, 1985. <<http://www.fao.org/Wairdocs/ILRI/x5488E/x5488e06.htm>>.
- Davies, D.H.K., Christal, A., Talbot, M., Lawson, H.M., Wright, G.M. (1997).** Changes in weed population in the conversion of two arable farms to organic farming. Proceeding. *Br. Crop Prot. Conf.: Weeds*: 1–3, 973–978.
- Deacon J. (2001).** The microbial world: The Nitrogen cycle and nitrogen fixation. Institute of cell and Molecular Biology, The University of Edinburgh. <<http://helios.bto.ed.ac.uk/bto/microbes/nitrogen.htm>>.
- De Boodt M., De Leenheer L. and Kirkham D. (1961).** Soil aggregate stability indexes and crop yields. *Soil Science*, 91:138-146.
- Del Pozo A. (1992).** Carbon nitrogen relations in cereals and legumes. University of Reading, United Kingdom. PhD Thesis.
- Del Pozo A. and Aronson J. (2000).** Ecophysiology of annual legumes. In: Sulas L. (ed.). Legumes for Mediterranean forage crops, pastures and alternative uses. CIHEAM-IAMZ, Zaragoza, pp. 223-230. Options Méditerranéennes, Ser. A 45 <<http://ressources.ciheam.org/om/pdf/c45/00600201.pdf>>.
- De-Polli H., Matsui E., Dobereiner J. and Salati E. (1977).** Confirmation of nitrogen fixation in two tropical grasses by $^{15}\text{N}_2$ incorporation. *Soil biology and biochemistry*, 9: 119-123.
- Dinesh R., Dubey R.P., Ganeshamurthy A.N. and Shyam Prasad G. (2000).** Organic manuring in rice-based cropping system: effects on soil microbial biomass and selected enzyme activities. *Current science*, 79(12): 1716-1720
- Dixon R.A., Achnine L., Kota P., Liu C.J., Reddy M.S.S. and Wang L. (2002).** The phenylpropanoid pathway and plant defense: a genomics perspective. *Molecular plant pathology*, 3: 371–390.
- Donahue S. (1998).** Legumes and soil quality. In: Soil Quality-Agronomy. USDA. Natural resources conservation service. Soil quality institute. *Technical note*, 6: 1-3.
- Durmur D., Pilbeam C. and Craigan J. (1990).** Use of weibull function to calculate cardinal temperature in faba bean. *Journal of experimental botany*, 41: 1423-1430.
- Ehrman T. and Cocks P.S. (1990).** Ecogeography of annual legumes in Syria: distribution patterns. *Journal of Applied Ecology* 27:578-591.
- Ehrman T. and Cocks P.S. (1996).** Reproductive patterns in annual legume species on an aridity gradient. *Vegetatio*, 122: 47-59.
- Entz M., Frick B., Wiens M. and Beavers R. (2006).** Increasing phosphorus availability through green manure, composted manure and rock phosphate. Organic Agriculture Center of Canada, Saskatoon <http://www.organicagcentre.ca/Docs/OACC_bulletins06/OACC_Bulletin17_phos_avail.pdf>.

- Evers G.W. (2003).** Principles of cool-season forage legume management. Texas A&M University Agricultural Research and Extension Center Verton, Texas. <<http://overton.tamu.edu/clover/cool/index.htm>>.
- Eyhorn F., Heeb M. and Weidmann G. (2004).** IFOAM Training manual for organic agriculture in the tropics. Theory, transparencies and didactic approach. Particularly: Chap. 4.5: Green Manures, pp. 124-130.
- Fairbrother T.E. and Rowe D.E. (1995).** Temperature and soil water effects on dormancy and mortality of subterranean clover seed. *Agronomy journal*, 87: 252-257.
- FAO. (2005a).** FAO statistical databases. <<http://apps.fao.org/page/collections>>.
- FAO, (2005b).** FAO statistical databases. <<http://www.fao.org/es/ess/top/commodity.html;jsessionid=5E31A868054E51B233BA0017C2B971CE?item=394&lang=en&year=2005>>.
- FAO (2005c).** FAO statistical databases, <<http://faostat.fao.org/site/291/default.aspx>>.
- Fara G., Franca A., Porqueddu C., Caredda S. and Roggero P.P. (1997).** Mediche e trifogli annuali autoriseminanti per usi foraggeri e non convenzionali. I. Adattamento e persistenza. *Rivista di agronomia*, 31: 1009-1018.
- Fairbrother T.E. and Rowe D.E. (1995).** Temperature and soil water effects on dormancy and mortality of subterranean clover seed. *Agronomy journal*, 87: 252-257.
- Fog K. (1988).** The effect of added nitrogen on the rate of decomposition of organic matter. *Biological review*, 63: 433-462.
- Fox G.A. (1989).** Consequences of flowering-time variation in a desert annual: Adaptation and history. *Ecology*, 70: 1294-1306.
- Fox R.H., Myers R.J.K. and Vallis I. (1990).** The nitrogen mineralization rate of legume residues in soil as influenced by their polyphenol, lignin and nitrogen content. *Plant and soil*, 129: 251-259.
- Fox J.E., Starcevic M., Kow K.Y., Burow and McLachlan J.A. (2001).** Nitrogen fixation : Endocrine disrupters and flavonoid signalling. *Nature*, 431: 128-129.
- Fried M., Danso S.K.A. and Zapata F. (1983).** The methodology of measurement of N₂ fixation by non-legumes as inferred from field experiments with legumes. *Canadian Journal of Microbiology*, 29: 1053-1062.
- Galloway, J., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W. and Seitzinger, S.P. (2004).** Nitrogen cycles: past, present and future. *Biogeochemistry*, 70:153-226.
- Garcia M.C., Marina M.L., Laborda F. and Torre M. (1998).** Chemical characterization of commercial soybean products. *Food Chemistry*, 62: 325-331.
- Gaskell M., Smith R., Mitchell J., Koike S.T., Fouche C., Hartz T., Horwath W. and Jackson L. (2006).** Soil fertility management for organic crops. University of California . Division of agriculture and natural resources. UC ANR Publication 7249. <<http://anrcatalog.ucdavis.edu/pdf/7249.pdf>>.
- Gepts P., Beavis W.D., Brummer E.C., Shoemaker R.C. and Stalker H.T. (2005).** Legumes as a model plant family. Genomics for food and feed report of the cross-legume advances through genomics conference. *Plant physiology*, 137(4): 1228-1235.
- Gordon N. (2005).** The nitrogen cycle, Human activities and the nitrogen cycle, Harmful effects of nitrogen deposition. The National Center for Atmospheric Reserch and the UCAR Office of Programs. <<http://www.ucar.edu/news/backgrounders/nitrogen.shtml>>.

- Graham P.H. and Vance C.P. (2003).** Legumes: importance and constraints to greater use. *Plant physiology*, 131: 872–877.
- Guet G. (1990).** Mémento d'agriculture biologique. 101 -103.
- Harrison A.J. (2003).** The nitrogen cycle: of microbes and men. *Visionlearning*, EAS-2 (4). < http://www.dlese.org/library/catalog_VL-EAS-2.4.htm >.
- HDRA The organic organization (1998).** Green manures/cover crops. http://www.gardenorganic.org.uk/pdfs/international_programme/GreenMan.pdf.
- Heichel G.H. and Barnes D.K. (1984).** Opportunities for meeting crop nitrogen needs from symbiotic nitrogen fixation. In: Bezdicsek D.E., Power J F., Keeney D. R. and Wright M L (eds.) *Organic farming: Current technology and its role in a sustainable agriculture*. ASA Spec. Publ. 46. ASA, CSSA, and SSSA, Madison, WI, pp. 49–59.
- Heichel G.H. and Vance C.P. (1979).** Nitrate-N and rhizobium strain roles in alfalfa seedling nodulation and growth. *Crop science*, 19: 512-518.
- Heichel G.H., Hardarson G., Barnes D.K. and Vance C.P. (1984).** Dinitrogen fixation, herbage yield, and rhizobial preference of selected alfalfa clones. *Crop science*, 24(6): 1093-1097.
- Heichel G.H., Vance C.P., Barnes D.K. and Henjum K.I. (1985).** Dinitrogen fixation and N and dry matter distribution during 4 year stands of birdsfoot trefoil and red clover. *Crop science*, 25: 101-105.
- Hekneby M., Antolin M.C. and Sanchez-Diaz M. (2001).** Cold response of annual Mediterranean pasture legumes. In: Delgado I. and Lloveras J. (eds.) *Quality in lucerne and medics for animal production*. CIHEAM-IAMZ, Zaragoza. *Options Méditerranéennes, Serie A 45*. pp.157-161
- Hesterman O.B. (1988).** Exploiting forage legumes for nitrogen contribution in cropping systems In: Hargrove W.L. (ed.) *Cropping strategies for efficient use of water and nitrogen*. ASA Spec. Publ. 51. ASA, CSSA, and SSSA, Madison, WI, pp.155–166.
- Hutchinson, C.M., McGiffen, M.E. (2000).** Cowpea cover crop mulch for weed control in desert pepper production. *Hort. Science*, 35: 196–198.
- Hue N.V. and Silva J.A. (2000).** Organic soil amendments for sustainable agriculture: organic sources of nitrogen, phosphorus and potassium. In: Silva J.A. and Uchida R.S. (eds.) *Plant nutrients management in Hawaii's soils*. CTAHR, University of Hawaii.
- ICARDA (1982).** Pasture, Forage and livestock Program. Annual Report 1982 (ICARDA, Aleppo, Syria).
- ILDIS: International Legumes Database and Information Service (2005).** Information about the family leguminosae. <<http://www.ildis.org/Leguminosae/>>.
- Jamea E.M. (2004).** Integration of annual self reseeding legumes into Mediterranean organic cropping systems. IAMB, Valenzano. Master thesis: Mediterranean organic agriculture, 351.
- Janzen H.H. and Radder G.D. (1989).** Nitrogen mineralization in a green manure-amended soil as influenced by cropping history and subsequent crop. *Plant and soil*, 120: 125-131.
- Jackson M.L. (1967).** Soil chemical analysis. Prentice Hall of India, New Delhi.
- Jackson M.L. (1958).** Soil chemical analysis. Prentice Hall, Englewood Cliffs.

- Jansen P.I. and Ison R.L. (1995).** Factors contributing to the loss of seeds from the seed-bank of *Trifolium balansae* and *Trifolium resupinatum* over summer. *Australian journal of ecology*, 20 (2): 248-256.
- Johnson G.A., Defelice M.S. and Helsel Z.R. (1993).** Cover crop management and weed control in corn (*Zea mays*). *Weed technology*, 7:425-430
- Jost J. (1998).** Cover crops and legumes. Kansas rural center: Sustainable agriculture management guides. <<http://www.kansasruralcenter.org/publications/covercrops.pdf>>.
- Kara E.E. (2000).** Effects of some plant residues on nitrogen mineralization and biological activity in soils. *Turkish journal of agriculture and forestry*, 24: 457-460.
- Killpack S.C. and Buchholz D. (1993).** Nitrogen in the environment. Nitrogen fixation. University of Missouri. <<http://muextension.missouri.edu/explore/envqual/wq0261.htm>>.
- Kirchmann H. and Bergquist R. (1889).** Carbon and nitrogen mineralization of white clover plants (*Trifolium repens*) of different age during aerobic incubation with soil. *Bodenkd*, 152: 283-288.
- Kohl D.H. and Shearer G.B. (1981).** The use of soil lightly enriched in ¹⁵N to screen for N₂ fixing activity in plants. *Plant and soil*, 60: 487-489.
- Köpke U. (1996).** Legume nitrogen in crop rotation: Reducing losses-increasing precrop effects. *Australian journal of agricultural research*, 8: 32-51.
- Kuepper G., Bachmann. J. and Thomas R. (2007).** Specialty Lettuce & Greens: Organic Production. <<http://attra.ncat.org/attra-pub/lettuce.html>>
- Lamond R.E., Whitney D.A., Bonczkowski L.C. and Hickman J.S. (1988).** Using legumes in crop rotations. Kansas State University. <<http://www.oznet.ksu.edu/library/crpsl2/L778.pdf>>.
- LaRue T.A. and Patterson R. (1981).** How much nitrogen do legumes fix? *Advances in Agronomy*, 34: 15-38.
- Lavorel S., Touzard B., Lebreton F.D. and Clément B. (1998).** Identifying functional groups for response to disturbance in an abandoned pasture. *Acta Ecologia*, 19: 227-240.
- Lesins K.A. and Lesins. I. (1979).** Genus *medicago* (Luguminosae): A taxogenetic study. W. Junk, The Hague, the Netherlands.
- Lerner R.B. and Dana M.N. (2001).** Growing cucumbers, melons, squash, pumpkins and gourds. Purdue University Cooperative Extension Service. <<http://www.hort.purdue.edu/ext/HO-8.pdf>>.
- Liebman, M., Davis, A.S. (2000).** Integration of soil, crop and weed management in low-external-input farming systems. *Weed Res.*, 40: 27-47.
- Lonchamp J.P. (2000).** Weed science and agronomy. Institut National de la Recherche Agronomique. Dijon. <http://www.dijon.inra.fr/hyppa/hyppa-a/noms_sca.htm#M>.
- Loges R., Ingwersen K., Kaske A. and Taube F. (2000).** Methodological aspects of determining nitrogen fixation of different forage legumes. In: Alföldi T., Lockeretz W. and Niggli U. (eds). Proceedings 13th International IFOAM Scientific Conference. IFOAM, 92.
- Magdoff, F., Van Es, H. (2000).** Building soils for better crops, 2nd Edition. Sustainable Agriculture Network, Handbook Series Book 4. Beltsville, MD, USA, 230pp.
- Mary B., Recous S., Darwis D. and Robin D. (1996).** Interactions between decomposition of plant residues and nitrogen cycling in soil. *Plant and soil*, 181: 71-82.

- Mazzoncini M., Bàrberi P., Cerrai D., Rinaudo V. and Belloni P. (2004).** Effects of green manure on soil nitrogen availability and crop productivity in a Mediterranean organic farming system. Presented at Eurosoil, Sept, 04-12. Freiberg, Germany.
- McGrath M.T. and Staniszewska H. (1996).** Management of powdery mildew in summer squash with host resistance, disease threshold-based fungicide programs, or an integrated program. *Plant Disease*, 80: 1044–1052.
- McGiffen Jr., M.E., Ehlers, J.D., Aguiar, J.L. (2000).** Introduction to organic horticulture colloquium. *Hort. Technology* ,10: 661–662.
- McKey D. (1994).** Advances in Legumes. Systematics 5: The Nitrogen factor, 241 pp. Royal Botanic Gardens Kew.
- Millennium Ecosystem Assessment (2005).** Ecosystems and Human Well-being: Synthesis. Island Press, Washington, DC.
- Merou T. and Papanastasis V.P. (2000).** Germination pattern of certain annual legumes in a sub-humid Mediterranean environment of Macedonia, Greece. In: Sulas L. (ed.). Legumes for Mediterranean forage crops, pastures and alternative uses. *CIHEAM-IAMZ, Zaragoza., Options Méditerranéennes* , Serie A 45 pp. 343-347.
- Mitchell J., Gaskell M., Smith R., Calvin F. and Koike S.T. (2000).** Soil management and soil quality for organic crops. University of California . Division of agriculture and natural resources. UC ANR Publication, 7249. <<http://anrcatalog.ucdavis.edu/pdf/7248.pdf>>.
- Miller R.M. and Jastrow J.D. (1992).** The role of mycorrhizal fungi in soil conservation. In: Bethlenfalvay G.J. and Linderman R.G. (eds.). Mycorrhizae in sustainable agriculture, 29-44. ASA Special Publication Number 54, Am. Soc. Agron., Madison, WI.
- Molinar R., Aguiar J., Gaskell M. and Maybemy K. (2000).** Summer squash production in California. Division of agriculture and natural resources. UC ANR. Publication, 7245. <<http://anrcatalog.ucdavis.edu/pdf/7245.pdf>>.
- Montanez A. (2000).** Overview and case study on biological nitrogen fixation: Perspectives and limitations. Case study B2. Prepared for FAO. <<http://www.fao.org/AG/aGL/agll/soilbiod/cases/caseB1.pdf>>.
- Mouaffak M.L. (1976).** L'expérience tunisienne dans le domaine de l'assolement céréales/légumineuses fourragères. Pp. 264-265. proceedings of third Regional Wheat Workshop, tunis, 1975. (Cimmyt: Mexico).
- Mulongoy K. (1995).** Technical paper 2: Biological nitrogen fixation. In: Tripathi B.R. and Psychas P. (eds). The AFNETA alley farming training manual - Vol 2: Source book for alley farming research. FAO, Rome. <<http://www.fao.org/Wairdocs/ILRI/x5546E/x5546e05.htm>>.
- Ndavidemi P.A. and Dakora F.D. (2003).** Review: legume seed flavonoids and nitrogenous metabolites as signals and protectants in early seedling development. *Functional plant biology*, 30: 729–745.
- Ngouajio M., McGiffen Jr M.E. and Hutchinson C.M. (2003).** Effect of cover crop and management system on weed populations in lettuce. *Crop Protection*, 22: 57–64.
- Neeson R. (2003).** Organic pumpkin production. New South Wales Agriculture <http://www.dpi.nsw.gov.au/data/assets/pdf_file/0019/113419/organic-pumpkin-production.pdf>.

- Nigel M. and Bennett S. (2001).** Plant genetic resources of legumes in the Mediterranean. Current plant science & biotechnology in agriculture. *Kluwer Academic Publisher*- Netherlands.
- Niggli, U. and Lockeretz W. (1996).** Development of Research in Organic Agriculture. In: Fundamentals of Organic Agriculture. Ed. By T.V. Ostergaard, Vol. 1, 11th *IFOAM Int. Scientific Conference, Copenhagen*: 9-24.
- Norman H.C., Cocks P.S., Smith F.P. and Nutt B.J. (1998).** Reproductive strategies in Mediterranean annual clovers: germination and hardseededness. *Australian journal of agricultural research*, 49: 973-982.
- Papastylianou I. (1999).** Estimation of nitrogen fixed by legumes in long-term vs. short term cropping system. *Agronomy journal*, 91: 329-334.
- Peterson T.A. and Russelle M.P. (1991).** Alfalfa and the nitrogen cycle in the corn belt. *Journal of soil and water conservation*, 46: 229-235.
- Phillips D.A., Center D.M. and Jones M.B. (1983).** Nitrogen turnover and assimilation during re-growth in *Trifolium subterraneum* L. and *Bromus mollis* L. *Plant physiology*, 71: 472-476.
- Piano E. and Talamucci P. (1996).** Annual self-regenerating legumes in Mediterranean areas. *Grassland science in Europe*, 1: 895-909.
- Puckridge D.W. and French R.J. (1983).** The annual pasture in cereal-ley farming systems of southern Australia: A review. *Agriculture, Ecosystems and Environment*, 9(3): 228-267.
- Quinlivan B.J. (1971).** Seed coat impermeability in legumes. *Journal of the Australian Institute of Agricultural Science*, 37: 283-295.
- Radaman M.S., Al-Fakhry A.K. and Al-Hasan A.M. (1978).** Some observations on the performance of annual medics in Northern Iraq. *Mesopotamia J. Agric.*, 13: 55-67.
- Rapport IDGC (1980).** Synthèse de la recherche et de l'exploitation du Medicago 1972 / 79. *Céréaliculture*, 13 : 18-26 .
- Raven P.H. (1973).** The evolution of Mediterranean floras. In: di Castri F. and Mooney H.A. (eds). Mediterranean-type ecosystems, origin and structure. Springer, Heidelberg : 213-224.
- Reeves D.W. (1994).** Cover crop and rotations. In: Hatfield J.L. and Stewart B.A. (eds.): Crops residue management. Advances in Soil Science. Lewis Publ., CRC, Boca Raton, FL: 125-158.
- Reinertsen S.A., Elliott L.F., Cochran V.L. and Campell G.S. (1984).** The role of available carbon and nitrogen in determining the rate of wheat straw decomposition. *Soil biology and biochemistry*, 16: 459-464.
- Rennie R.J. and Rennie D.A. (1983).** Techniques for quantifying N₂ fixation in association with non-legumes under field and greenhouse conditions. *Canadian journal of microbiology*, 29: 1022-1035.
- Rhoades J.D. (1982).** Soluble salts. In: Page A.L. (ed.) Methods of soil analysis, Part2, chemical and microbiological properties. *Agronomy*, 9: 149-157.
- Roberts E.H., Summerfield R.J., Ellis E.H., Craufurd P.Q. and Wheeler T.R. (1997).** The induction of flowering. In: Wien H.C. (ed). The physiology of vegetable crops. CAB International, pp. 69-99.

- Roberts P. and Kucharek T. (2007).** Florida plant disease management guide: squash. University of Florida. Department of Plant Pathology. <http://edis.ifas.ufl.edu/PG055#FOOTNOTE_1>
- Rochon J.J., Doyle C.J., Greef J.M., Hopkins A., Molle G., Sitzia M., Scholefield D. and Smith C.J. (2004).** Grazing legumes in Europe: a review of their status, management, benefits, research needs and future. *Grass and forage science*, 59: 197–214.
- Roth G.W. (1996).** Crop rotation and conservation tillage. Pennsylvania State University. Conservation tillage. Series 1. <<http://cropsoil.psu.edu/extension/ct/uc124.pdf>>.
- Rossiter R.C., Maller R.A. and Parkes A.G. (1985).** A model of changes in the composition of binary mixtures of subterranean clover strains. *Australian journal of agricultural research*, 36: 119-143.
- Schenck S. (2003).** Soil incorporation of cover crop biomass: effects on soil microorganisms and nitrogen levels. Hawaii Agriculture Research Center. Diversified crops report, pp. 23.
- Sanchez-Diaz M. (2001).** Adaptation of legumes to multiple stresses in Mediterranean-type environments. In: Delgado I. and Lloveras J. (eds.). Quality in Lucerne and medics for animal production. CIHEAM-IAMZ, Zaragoza, pp.145-151. Options Méditerranéennes, Ser. A 45. <<http://ressources.ciheam.org/om/pdf/a45/01600074.pdf>>.
- Schmid O., Strasser F., Gilomen R. and Meili E. (1991).** Biologischer Landbau: Lehr- und fachbuch für landwirtschaftliche Schulen und die Praxis, IMZ, Landwt. Lehrmittelzentrale, 3052, Zollikofen, CH.
- Semple E.C. (1928).** Ancient Mediterranean agriculture: II. Manuring and seed selection. *Agricultural History*, 2: 129-156.
- Sheaffer C.C. and Lake A.W.H (1997).** Legumes in cropping systems. In: Buchanan-Smith J.G. (ed.). Approaches in midwest United States and Southern Australia. Proc. 18th Int. Grassld. Congr., Winnipeg, MB, and Saskatoon, SK, Canada. 8–17 June 1997. Association Management Centre, Calgary, AB, Canada, pp. 349–354.
- Sheaffer C.C., Simmons S.R. and Schmitt M.A. (2001).** Annual medic and berseem clover dry matter and nitrogen production in rotation with corn. *Agronomy Journal*, 93: 1080-1086.
- Shennam C. (1992).** Covers crops, nitrogen cycling, and soil properties in semi-irrigated vegetable production systems. *HortScience*, 27:749-753.
- Silva J.A. and Uchida R. (2000).** Biological nitrogen fixation, Nature's partnership for sustainable agriculture production. In: Silva J.A. and Uchida R. (eds.). Plant nutrient management in Hawaii's soils. Approaches for tropical and subtropical agriculture, College of tropical agriculture and human resources, University of Hawaii at Manoa, pp. 121-126. <<http://www.ctahr.hawaii.edu/oc/freepubs/pdf/pnm13.pdf>>
- Smil V. (2000).** Cycles of life: Civilization and the biosphere. Scientific American library, New York.
- Steduto P., Todorovic M. and Sisto L. (2004).** Agro-Meteorological Bulletin. CIHEAM- IAM- Bari, Valenzano, Italy, n°33-34-35-36.
- Stopes C. and Millington S. (1995).** Dry matter and nitrogen accumulation by three leguminous green manure species and the yield of the following wheat crop in an organic production system. *Agriculture, Ecosystems and Environment*, 57: 189-196.

- Sulas L., Franca A. and Caredda S. (2000).** Persistence and regeneration mechanisms in forage legumes. In: Sulas L. (ed.). Legumes for Mediterranean forage crops, pastures and alternative uses. CIHEAM-IAMZ, Zaragoza, pp. 331-342. Options Méditerranéennes, Ser. A 45.
- Sullivan P. (2003).** Overview of cover crops and green manures. Fundamentals of sustainable agriculture ATTRA- National sustainable agriculture information services. <<http://attra.ncat.org/attra-pub/covercrop.html>>.
- Taiz L. and Zeiger E. (2002).** Plant physiology. 3rd ed. University of California, Santa Cruz, and University of California, Los Angeles, with contributors. Chapter 3, topic 23.5.
- Taylor G.B. (1984).** Effect of burial on the softening of hard seeds of subterranean clover. *Australian journal of agricultural research*, 35: 201-210.
- Taylor G.B., 1996.** Effect of the environment in which seeds are grown and softened on the incidence of autumn seed softening in two species of annual medics. *Australian Journal of Agricultural Research*, 47: 141-159.
- Taylor G.B. and Ewing M.A. (1998).** Effect of depth of burial on the longevity of hard seeds of subterranean clover and annual medics. *Australian journal of Experimental agriculture*, 28: 77-81.
- Tisdall J.M. (1994).** Possible role of soil microorganisms in aggregation in soils. In: Robson A D., Abbott L K. and Malajczuk N. (eds.). Management of mycorrhizas in agriculture, horticulture and forestry. Kluwer Academic Publishers, Dordrecht, pp. 115-121.
- Teasdale J.R. (1996).** Contribution of cover crops to weed management in sustainable agricultural systems. *Journal of Production Agriculture*, 9: 475-479.
- Tesi R. (1990).** Cucurbitaceae. In: Bianco V.V. and Pimpini F. (coords.). *Orticultura*. Patron, Bologna, pp. 622-636.
- Ten Holte L. and Van Keulen H. (1989).** Effects of white clover and red clover as a green crop on growth, yield and nitrogen of sugar beet and potatoes. *Developments in plant and soil sciences*, 37: 16-24
- Thorup-Kristensen K. and Bertelsen M. (1996).** Green manure crops in organic vegetable production. In: Kristensen N.H. and Høeg-Jensen H. (eds.). New research in organic agriculture, Proceedings from the 11th international scientific IFOAM conference, Copenhagen, pp. 75-79.
- Thorup-Kristensen K. and Nielson N.E. (1998).** Modelling and measuring the effect of nitrogen catch crops on the nitrogen supply for succeeding crops. *Plant and Soil*, 203: 79-89.
- Thorup-Kristensen K. and Grevsen K. (1999).** An organic vegetable crop rotation self-sufficient in nitrogen. *Nordisk Jordbrugsforskning*, 81 (3):206-214.
- Thorup-Kristensen K. (2001).** Are differences in root growth of nitrogen catch crops important for their ability to reduce soil nitrate-N content, and how can this be measured? *Plant and Soil*, 230: 185-195.
- Thorup-Kristensen, Kristian (2002).** Six years results from an organic vegetable crop rotation aimed at self-sufficiency in nitrogen. [oral] Presentation at XXVIth International Horticultural Congress & Exhibition (IHC2002), Toronto, Canada, August 2002.

- Tilman D. (1999).** Global environmental impacts of agricultural expansion: the need for sustainable and efficient practices. *Proceedings of the National Academy of Science of the United State of America*, 96: 5995-6000.
- Unkovich M. and Pate J.S. (2001).** Assessing N₂ fixation in annual legumes using ¹⁵N natural abundance. In : Unkovich M., Pate NcNeill A. and Gibbs D.J. (eds). Stable isotope techniques in the study of biological processes and functioning of Ecosystem. *Kluwer Academic Publisher*, Dordercht, pp. 103-118.
- Vance C.P., Graham P.H. and Allan D.L. (2000).** Biological nitrogen fixation. Phosphorus: a critical future need. In: Pedrosa F.O., Hungria M., Yates M.G., Newton W.E. (eds). Nitrogen fixation: from molecules to crop productivity. *Kluwer Academic Publishers*, Dordrecht, pp. 506–514.
- Vantine M. and Verlinden S. (2003).** Maintaining soil fertility under an organic management system. West Virginia University. SARE Northeast region: Sustainable agriculture research and education program. <<http://www.wvu.edu/~agexten/farmman2/organic/soilfert.pdf>>.
- Viggiani P. (1990).** “Erbe spontanee e infestanti: tecniche di riconoscimento” dicotiledoni. Bayer. 271 p.
- Waskom R.M. (1994).** Best management practices for nitrogen fertilization. Colorado StateUniversity. <http://www.wcainfonet.org/servlet/BinaryDownloaderServlet?filename=1068471970371_cl3.pdf&refID=119912>.
- Whiting D., Wilson C. and Card A. (2003).** Cover crops and green manure crops. Colorado master garden .Colorado state university. < <http://cmg.colostate.edu/gardennotes/244.pdf> >.
- Willson A. (2005).** Types of nitrogen fixation. The world of nitrogen fixation series. Part 1-. *Australian organic journal*, Autumn: 23. <http://www.bfa.com.au/files/AOJ%20iss61_p23.pdf>.
- Wright S.F. and Upadhaya A. (1996).** Extraction of an abundant and unusual protein from soil and comparison with hyphal protein from arbuscular mycorrhizal fungi. *Soil science*, 161: 575-586.
- Zohary M. (1987).** Flora Palaestina. Part two. Text platanaceae to umbelliferae. The Israel Academy of Science and Humanities, *Goldbergs Press, Jerusalem*, pp. 13-209.